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13. ABSTRACT (Maximum 200 words) To assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health safety and performance, the PBRC performs the following eight research tasks: 1) Clinical Laboratory for Human and Food Samples performs laboratory analysis of samples from studies conducted by the U.S. Army Research Institute of Environmental Medicine (USARIEM) and at PBRC in Tasks 4 and 8. 2) Stable Isotope Laboratory performs analyses to measure the energy expenditure and body composition of soldiers during prolonged field exercise and at PBRC in Tasks 4 and 8. 3) Stress, Nutrition and Mental Performance Laboratory continues multidisciplinary basic research studies of the interactions of stressors and nutrition on mental performance parameters in an animal model. This lab collaborates with Task 7 where samples are sent to evaluate immune function in the stressed animal model. 4) Stress Nutrition and Work Performance uses human subjects to develop nutritional strategies to improve physical performance under intense physical stress and to collaborate with Task 7, sending samples for analysis to link muscular fatigue to decrements in immune function. 5) Nutrient Database Integration Laboratory supports the Military Nutrition Division and PBRC research studies by providing dietary intake and analysis support. 6) Enhancing Military Diets has expand the Armed Forces Recipe file with healthier recipes and has developed research targeted at garrison health promotion. 7) Stress Nutrition and Immune Function Laboratory provides special tests of immune function in collaboration with Tasks 3 and 4 to evaluate immunologic change in humans and in a rat model. 8) Metabolic Unit Project allows new inpatient protocols to address specific issues in nutritional interactions with stress which affect performance and immune function.					
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FOREWORD

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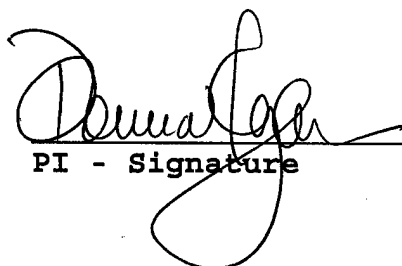
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ANNUAL REPORT
US ARMY GRANT #DAMD 17-97-2-7013
July 1, 1998 - June 30, 1999

Introduction

On April 1, 1997, Grant #DAMD 17-97-2-7013 was awarded to Pennington Biomedical Research Center (PBRC) to address the following **hypothesis: Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness.**

The goal of this research is to assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health, safety and performance.

Technical Objective

This research continues the research relationship between the PBRC and USARIEM over a five year period. Those research relationships were established under prior cooperative agreements, #DAMD 17-88-Z-8023, "The effect of food, diet and nutrition on military readiness and preparedness of military personnel and dependents in a peace time environment," and #DAMD 17-92-V-2009, "Military nutrition research: six tasks to address medical factors limiting soldier effectiveness."

The project allows for the continuation of the Clinical Laboratory for Human and Food Samples, Stable Isotope Laboratory, Menu Modification Project, and Nutritional Neuroscience Laboratory, all of which were initiated under Grant #DAMD 17-88-Z-8023. The project also expands the scope of research to allow for studies in humans of stress, nutrition and work performance, and for studies in humans and animals to evaluate the relationship of stress, nutrition and immune function. The grant provides a nutrient database laboratory. The project also allows for the utilization of the PBRC's inpatient metabolic unit for a study designed by USARIEM investigators as detailed in the Metabolic Unit Project section.

Military Significance and Relevance to USARIEM Needs

The Stable Isotope and Clinical Laboratory methodologies are critical components of in-house military nutrition research of the U.S. Army Research Institute of Environmental Medicine. These extramural projects provide critical capabilities that do not exist in house, but are needed to fulfill the Army Surgeon General's responsibility to provide nutritional research support to the DOD and Nutrition RDT&E Program.

The Nutritional Neuroscience Laboratory expands our knowledge of the effects of stress and sleep deprivation and explores the ameliorative effects and mechanisms of action of dietary-induced alterations in behavior and cognitive function. Advances in this knowledge are the basis

for developing safe and effective nutritional strategies to sustain and enhance soldier performance under conditions of environmental or operational stress. The project also provides insight into the roles of corticotrophin releasing factor (CRF) and locus coeruleus (LC) noradrenergic mechanisms in mediating anxiety in rats exposed to restraint stress.

The Menu Modification Project fulfills military needs to promote health, maintain readiness and sustain soldier performance. The Nutrient Database Integration Laboratory supports USARIEM projects assessing food intake in the field.

The Metabolic Unit Project fulfills military need for an inpatient site for performance of specialized research utilizing the body composition assessment, energy expenditure assessment, metabolic kitchen services, and clinical laboratory expertise of the PBRC.

This annual report describes progress during the second year of the grant. Discussions of individual projects funded under this grant follow.

- In the 5th quarter of the grant we purchased with grant funds computer equipment and food preparation equipment to support grant activities. The correspondence detailing this is found in the Appendix.
- In the 6th quarter we received correspondence documenting acceptability of our annual report for year one (see Appendix). We also provided information regarding our animal use to Major Steven Tobias in Natick and Major David Ruble of the Animal Care and Use Review Division.
- In the 7th quarter of the grant we executed a modification for incremental funding (see Appendix). We were also visited by project officers 2/22/99-2/23/99. The visitors included Dr. Harris Lieberman, Major Vicky Thomas, Colonel Richard Lynch, Colonel Sue Standage, Captain Mark Kellogg and Captain Mary Chamberlain. The agenda is found in the Appendix. On 2/2-2/5/99 Dr. Steve Smith attended a workshop sponsored by the Committee on Military Nutrition Research and presented the grant's prior research on sleep deprivation, performance and caffeine.
- In the 8th quarter we assisted USAMRMC with the preparation of a fact sheet. This is attached in the Appendix. We corresponded with our contracting officer, Michael A. Younkers, regarding a budget shortfall and submitted a revised budget (see Appendix). Also in the last quarter, Dr. Alana Cline notified us of her plans to leave the Pennington Center effective 7/30/99 (see Appendix).

The eight tasks performed under this project are listed and described below.

I. Clinical Laboratory for Human and Food Samples

A. Introduction

The Clinical Research Laboratory, which functions as a support laboratory for the U.S. Army's nutritional research program, continued to receive and analyze samples for the Army in 1998-1999. The number of studies for which the laboratory performed tests increased from last year. There were a total of nine studies for which the laboratory did testing.

The laboratory continues to offer a broad spectrum of analytical tests in support of army research. In addition to the standard tests performed in the past a few new methods were put on line and new instrumentation was obtained (see below).

B. Body

In the past year the laboratory replaced one medical technologist (Deonne Bodin) with another (Stacey Roussel). In the Food Analysis Laboratory, because of the departure of Dianne Ratcliffe and loss of funding for her job, two medical technologists are now rotating between the Clinical Research Laboratory and the Food Analysis Laboratory. Both of these technicians are spending one day a week in the food laboratory and four days in the clinical lab.

New instrumentation and tests were put on-line in 1998-9. A new method of fecal processing for nitrogen analysis was developed. This was necessary because the old $\text{H}_2\text{SO}_4/\text{CuSO}_4/\text{H}_2\text{O}_2$ digestion would not give reliable results on the new nitrogen analyzer. The new digestion is in a sealed ampule with hydrochloric acid and phenol. Recovery results were very good for this method.

New methods were put in operation for myoglobin, C Reactive Protein, IL-1 β , IL-6, and TNF- α on the DPC Immulite. The tests were all validated and are functioning very well. The sensitivity limits for CRP and IL-1 β were decreased so that normal levels could be detected. In spite of this, the majority of IL-1 β results were below our detection limit in the Eccentric Exercise Study(EES) study. Our laboratory was approved by Abbott Laboratories as a site for pre-release use of their new plasma homocysteine kit for the Abbott IMx. We have decided to use this method for our samples. A new instrument was obtained from Abbott to help us perform the large number of samples awaiting analysis. Results on precision and linearity were excellent.

A method for C peptide on the DPC Immulite was also set up and validated.

A method for the analysis of carotenoids is being developed by HPLC. The method also allows for the simultaneous analysis of vitamins A and E. Some difficulties have been encountered but the method appears to be promising.

Data for new methods is included in the Appendix.

Studies in for which testing was performed this past year include Ranger 4, SAFS-6, "Post Exercise Nutrient Supplementation study (PENS), Sargent Major's Academy, Combat Army Support Hospital (CASH), Ranger Regiment Nutritional Survey, "Effects of Immune Egg Protein and Antioxidants on Muscle Soreness and Strength after Eccentric Exercise" (EES), "Effects of Repeated Dosings of Caffeine on Vigilance", and Mangoday.

Testing for Ranger 4 has been completed for TSH, T3 free and total, T4 free and total, testosterone, growth hormone, DHEAS, folate, BHBA, NEFA, lactate, and chemistry panel. Testing for vitamin A, vitamin E, and carotenoids is awaiting development of a method for carotenoids.

Testing for SAFS-6 has been completed for the chemistry panel only.

Analyses were performed for Dr. Hal Goforth of the Navy for a pilot study in San Diego. Testing included a chemistry 26 panel, glucose, CK, and amino acids (leucine, alanine, glutamine, and glutamic acid). The first two shipments of samples for the actual study were received. Chemistry and analyses have been completed on the first shipment. Amino acids are awaiting some trouble-shooting on the method. Samples from the second shipment arrived but have not been analyzed as of this date. These will be forthcoming.

Work was performed on the study titled "Effects of Immune Egg Protein and Antioxidants on Muscle Soreness and Strength after Eccentric Exercise" (EES). Tests completed include IL-1, TNF, IL-6, CRP, Vitamin C, bilirubin, albumin, CK, Alkaline Phosphatase, and LDH. Tests still pending include total antioxidant capacity, vitamin C, myosin heavy chain fragments (MHCF), and vitamin E. Myosin heavy chain fragment test has not been set up yet. To date, we have been unsuccessful in finding a commercially available assay. A method needs to be developed for urinary myoglobin also.

Analyses for salivary melatonin and caffeine were performed for Harris Lieberman's study "Effects of Repeated Dosings of Caffeine on Vigilance" (our code name is Caffeine and Sleep Deprivation (CSD). These have been completed and are awaiting shipment. Salivary melatonin analyses are also in progress for the Mangoday study. It should be noted that we received very good and clean samples for this study.

Homocysteine analyses were completed for the Ranger Regiment Nutrient Survey, Combat Support Hospital (CASH), and Sergeant Major's Academy. Results for CASH and Sergeant Major's have been returned and the Ranger Regiment Nutrient Survey results will be sent shortly.

A method of direct access to their data by the army is being investigated. Possible answers to the request of the army include a terminal with Meditech software which would allow them to view completed results on samples for their studies. A problem with this is that it would

be on a sample by sample basis and no compiled results would be obtainable. The other alternative is to give the army access to a special database set-up by computer administration upon completion of the results for a study. This would allow for direct access to compiled data; however, methods of ensuring the safety of the Pennington database would have to be a priority. Approval by the Pennington administration would have to be obtained for either of these options to be implemented.

Key Research Accomplishments

- Developed and put in use new tests for the analysis of myoglobin C reactive protein, interleukin 1 β , interleukin 6, tumor necrosis factor, homocysteine, and C peptide.
- Performed analyses for the Army on nine studies including EES, PENS, Ranger 4 SAFS6, Sargent Majors Academy, Ranger Regiment Survey, CASH, Caffeine and Sleep Deprivation and Mangoday.
- Evaluated and established a new method for fecal digestion for nitrogen analysis using HCl and phenol.
- Worked on a new method of analysis of carotenoids.

C. Conclusions

The clinical laboratory performed testing for nine studies and worked on methods for fecal digestion with hydrochloric acid/phenol for nitrogen analysis, homocysteine, carotenoids, myoglobin, C Reactive Protein, IL-1 β , IL-6, TNF- α , and C peptide.

D. References

None applicable.

II. Stable Isotope Laboratory

A. Introduction

The research conducted by the Stable Isotope Laboratory is in the area of energy and water requirements, and changes in body water, of soldiers, often under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water (DLW) technique, which involves oral administration of water labeled with the stable isotopes, ^2H and ^{18}O . Saliva and urine samples are then obtained for periods of 4-14 days, longer with redosing. Water intake can be determined using only the ^2H labeled water. The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals (1). The method is based on the premise that after a loading dose of $^2\text{H}_2^{18}\text{O}$, ^{18}O is eliminated as CO_2 and water, while

deuterium is eliminated from the body as water. The rate of CO₂ production, and, hence, energy expenditure, is calculated from the difference of the two elimination rates. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of ²H₂¹⁸O, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries. The doubly labeled water method has been extensively validated in humans under controlled settings (2), but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations (3). This is a particular problem with studies such as the Ranger Training Studies (4), in which soldiers are moved to different parts of the country during the study. Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Hydration status is another main focus for some Army studies. Using the cheaper and more readily available deuterium tracer, either changes in total body water (5,6) can be followed during a study, or water turnover (intake) (7,8) can be measured during a study.

One advantage of the DLW method is that it uses stable isotopes so there is no radiation exposure. The method uses two heavy isotopes of water, which are naturally occurring in food and water. There are no known side effects of either isotope at the doses given in DLW studies and has been used extensively to study energy expenditure during pregnancy (10,11) lactating women (12), and infants for measurement of energy expenditure and human milk intake (13-15).

The Stable Isotope Lab was involved in several Army research projects during the current year. These are described below.

B. Body

Stable isotope studies were completed for 3 studies and a fourth large study was begun. The first study employing doubly labeled water was entitled: "Effects Of Tray Ration Consumption During A 63-Day Marine Field Exercise." The 2nd study completed was a study conducted in June, 1998 in which Norwegian Rangers underwent an intense training exercise under harsh environmental conditions. The 3rd study was an Infantry Officer Training Course, conducted at Quantico March, 1999.

Energy expenditures for Marines undergoing a 63-day field exercise were completed and reported in the 3rd quarter of the year. The mean energy expenditure for the 3 dosing periods of this construction mission was 3600±560 kcal/d, 3220±690 kcal/d and 3270±770. These values are typical compared to other field training exercises. The energy expenditure was highest during the first phase, most likely because of the physically demanding tasks of unloading equipment and

supplies coupled with the digging of the foundation during the first week. There was no difference between the two ration groups. The Average energy expenditure of 3330 kcal/d was less than the 3600 kcal/d Nutritional Standard for Operation Rations, suggesting that this standard is adequate for moderately active Marines in normal field missions. Those consuming the T Ration were in greater negative energy balance (-784 kcal/d) compared to those consuming the B Ration (-448 kcal/d) over the course of the study. The overall water turnover calculated from the deuterium turnover rates was 5.7 ± 1.0 L/d. The more active construction engineers had a significantly greater water turnover rate than administrative and support personnel (6.0 ± 0.9 vs 5.0 ± 0.8 L/d).

Isotope analyses and calculations were completed for the Norway '98 study. Baseline isotope shifts (D_2O and $H_2^{18}O$) for the two subjects not receiving isotope were used to adjust isotope enrichments in the subjects receiving the $D_2^{18}O$. Energy expenditure was calculated by calculating elimination rates of deuterium and ^{18}O by linear regression, using multiple time points. Since these soldiers received very little food, and hence relied largely on their fat stores for energy, we used a calculated RQ of 0.75 for conversion of CO_2 elimination to energy expenditure. The mean energy expenditure in this study was very high, 6250 ± 770 kcal/d, 500 kcal/d higher than was observed in the Norway '97 study of 5650 ± 800 kcal/d.

Isotope analyses are nearly complete for an Infantry Officer Training Course, conducted at Quantico March, 1999. All samples from the field study have been received and logged in the stable isotope lab. Sample cleanup has been completed. Oxygen-18 and deuterium Isotope analyses are complete for the placebo group, and nearly complete for the labeled subjects. The isotope baseline shift in the undosed placebo group is shown in the table below and in a figure in the Appendix. These baseline shifts are being used to adjust the enrichments in the subjects receiving the doubly labeled water.

		Subject			
Date	Time	1	3	7	10
<u>¹⁸O, o/oo</u>					
03/03/99	0	-3.24	-3.53	-3.25	-4.03
03/04/99	1	-2.84	-3.46	-2.65	-3.85
03/06/99	3	-3.60	-3.61	-3.44	
03/08/99	5	-3.73	-3.68	-3.73	
03/10/99	7	-3.66	-3.68	-3.28	-4.38
03/12/99	9		-3.70	-3.03	-4.41
03/13/99	10		-3.23	-3.30	-3.98
<u>Deuterium, o/oo</u>					
03/03/99	0 HR	-30.64	-27.08	-32.11	-39.25
03/04/99	1 DAY	-28.98	-39.15	-32.18	-35.86
03/06/99	3 DAY	-41.47	-35.66	-27.18	
03/08/99	5 DAY	-37.94	-39.43	-49.15	

03/10/99	7 DAY	-41.70	-35.16	-40.15	-34.88
03/12/99	9 DAY		-37.87	-39.50	-45.44
03/13/99	10 DAY		-30.00	-40.80	-35.36

Key Research Accomplishments

- Conducted stable isotope measures in a 63 day study of Marines undergoing a construction mission. Major outcome variables examined were total daily energy expenditures, energy deficits and water intake and hydration status
- Conducted a study of Norwegian Rangers undergoing a vigorous training exercise under adverse conditions.
- Carried out doubly labeled water studies in an Infantry Officer Training Course, conducted at Quantico.

Reportable Outcomes

A Technical report is being prepared for the Marine construction mission: Chapter 6. Energy expenditure, water turnover and hydration status. W.J. Tharion, C.J. Baker-Fulco, R.W. Hoyt and J.P. DeLany.

C. Conclusions

Average total daily energy expenditure during the Marine construction mission was 3330 kcal/d. Construction engineers expended more energy than administrative and support personnel. Current ration policy (NSOR) provides sufficient energy to meet the demands of combat engineers performing this type of mission. Negative energy balances were greatest for construction engineers consuming T Rations. As time progressed these negative energy balances increased. While the Marine leadership continually stressed the importance of fluid consumption, the difficulty of individuals obtaining and consuming sufficient fluids while away from base camp was a problem. The education of every soldier and marine on the importance of fluid consumption and the consequences of hypohydration needs to continue.

The energy expenditures reported for the Norwegian Ranger Training studies are some of the highest reported energy expenditures we have observed in military personnel 6250 ± 770 kcal/d this year, 500 kcal/d higher than was observed in the Norway '97 study of 5650 ± 800 kcal/d. The only energy expenditures approaching this level were those observed in the Marine Crucible event and the "Mountain Class" phase of the Ranger Training studies.

D. References

1. Lifson, N. Theory of use of the turnover rates of body water for measuring energy material balance. *J. Theoret. Biol.* 12:46-074, 1966.
2. Schoeller DA. Measurement of energy expenditure in free-living humans by using doubly labeled water. *J Nutr* 1988;118:1278-89.
3. DeLany James P., Dale A. Schoeller, Reed W. Hoyt, E. Wayne Askew, and Marilyn A. Sharp. Field Use of D₂¹⁸O to Measure Energy Expenditure of Soldiers at Different Energy Intakes. *J Appl Physiology* 1989;67:1922-9.
4. Hoyt, R.W., R.J. Moore, J.P. DeLany, K.E. Friedl, and E.W. Askew. Energy balance during 62 days of rigorous physical activity and caloric restriction. *FASEB J* 7:A726, 1993 (Abs).
5. Schoeller, DA, E van Santen, DW Peterson, W Dietz, J Jaspen and PD Klein. Total body water measurement in humans with ¹⁸O and ²H labeled water. *Am J Clin Nutr* 1980;33:2686-93.
6. Lyons, T.P., S.R. Muza, A. Cymerman, and J.P. DeLany. Total body water and plasma volume responses to high altitude acclimatization and deacclimatization. *FASEB J* 8:A288, 1994 (Abs).
7. Fjeld C.R., K.H. Brown, and D.A. Schoeller. Validation of the deuterium oxide method for measuring average daily milk intake in infants. *Am J Clin Nutr* 1988;48:671-9.
8. Jones, T.E., R.W. Hoyt, J.P. DeLany, R.L Hesslink, E.W. Askew. A comparison of two methods of measuring water intake of soldiers in the field. *FASEB J* 7:A610, 1993 (Abs).
9. Lyons, T.P., B.J. Freund, A.J. Young, M.N. Sawka, S.R. Muza, A. Cymerman, C.R. Valeri and J.P. DeLany. Erythrocyte infusion effects on fluid redistribution at high altitude. *Faseb J* 1995, 9:A650.
10. Heini A, Schutz Y, Diaz E, Prentice AM, Whitehead RG, Jéquier E. Free-living energy expenditure measured by two independent techniques in pregnant and nonpregnant Gambian women. *Am J Physiol* 1991;261:E9-E17.
11. Goldberg GR, Prentice AM, Coward WA, Davies HL, Murgatroyd PR, Wensing C, Black AE, Harding M, Sawyer M. Longitudinal assessment of energy expenditure in pregnancy by the doubly labeled water method. *Am J Clin Nutr* 1993;57:494-505.

12. Goldberg GR, Prentice AM, Coward WA, Davies HL, Murgatroyd PR, Sawyer MB, Ashford J, Black AE. Longitudinal assessment of the components of energy balance in well-nourished lactating women. *Am J Clin Nutr* 1991;54:788-98.
13. Roberts SB, Coward WA, Ewing G, Savage J. Effect of weaning on accuracy of doubly labeled water method in infants. *Am J Physiol* 1988;254:R622-R627.
14. Fjeld CR, Brown KH, Schoeller DA. Validation of the deuterium oxide method for measuring average daily milk intake in infants. *Am J Clin Nutr* 1988;48:671-679.
15. Butte NF, Wong WW, Patterson BW, Garza C, Klein PD. Human-milk intake measured by administration of deuterium oxide to the mother: a comparison with the test-weighing technique. *Am J Clin Nutr* 1988;47:815-821.

III. Stress, Nutrition and Mental Performance

A. Introduction

The objective of research conducted in this Task is to investigate mechanisms mediating stress-induced behavior in rodents, with the goal of identifying nutritional interventions that prevent, or ameliorate, some of the negative aspects of stress. If we are successful using animal models, the information will be transferred to investigators working with human subjects for evaluation of the dietary treatment in a clinical trial. In animal studies we are able to measure behavior following exposure to a controlled mixed psychological and physical stressor, such as restraint or sleep deprivation, and then analyze tissues for expression of specific proteins, neurotransmitters or hormones. The group that works on this project includes individuals with a range of scientific expertise, facilitating studies that include behavioral and subcellular measures of the stress response in the same animal. We have identified several models of stress that lead to reliable and repeatable changes in behavior of rats. The two that we use most frequently are repeated restraint stress (3 hours of restraint for 3 days) and REM sleep deprivation for periods of up to 96 hours. Both of these stressors cause changes in energy balance and cognitive function and induce anxiety. In the studies described below we have investigated the physiological and biochemical changes that accompany these responses in an effort to elucidate the underlying mechanisms.

B. Body

The Effect of Repeated Restraint Stress on Post-Stress Energy Metabolism

Jun Zhou, Xiaolang Yan, Leigh Anne Howell, Jacob Simpson, Tiffany Mitchell, Bradley Youngblood, Ruth B.S. Harris

In our previous experiments we have shown that rats exposed to repeated restraint stress (3 hours restraint stress for 3 consecutive days) experience a temporary reduction in food intake

and a sustained reduction in weight (Rybkin et al., 1997; Harris et al., 1998). The initial weight loss was accounted for by lean tissue when measured one day after the end of stress. However, the maintained reduction in weight was accounted for by both lean and fat tissue (Harris et al., 1998). These results indicated that restraint had sustained effects on tissue metabolism even after the stress was terminated. Oral glucose tolerance tests indicated that insulin insensitivity caused by feeding a high fat diet was improved in restrained rats but muscle glucose uptake was not increased and adipocyte glucose transport was inhibited by restraint stress, when measured one day after the end of the last restraint (Zhou et al., 1999). Therefore, we investigated the effect of repeated restraint on liver glucose and fatty acid metabolism.

Male Sprague Dawley rats were fed a 40% kcal fat diet for 10 days then were divided into 3 groups: control, restrained or non-restrained rats pair-fed to restrained rats. One day after the end of repeated restraint hepatocytes were isolated from each rat and glucose transport was measured. Glucose transport was increased in hepatocytes from both restrained and pair-fed rats, compared with controls (see Figure 1), but there were no differences in glucose or fatty acid oxidation or incorporation into lipids (data not shown). Measurement of glycogen synthesis in slices of liver tissue from rats exposed to an identical experimental paradigm indicated increased rates of basal glycogen synthesis in liver from restrained, but not pair-fed rats (Figure 2). These results indicated that there were tissue specific changes in metabolism such that glucose uptake was inhibited in adipose tissue but short-term energy storage of glucose as glycogen was increased in the liver. The increased liver glucose transport may be the major reason for an increased whole body glucose uptake, demonstrated as an improved insulin sensitivity in response to an oral glucose challenge. Also, the increased liver glycogen content during the post stress period might serve as an erroneous satiety signal in restrained rats, inhibiting compensatory hyperphagia during the post stress period.

Although we had identified metabolic changes during the post-stress period, the trigger for these changes had not been identified. Evidence for involvement of β -adrenergic receptors in tissue energy utilization is well established (Germack et al., 1997). Under conditions of stress the sympathetic nervous system is activated, norepinephrine and epinephrine are released from the adrenal and cause different effects on different organs, depending on the type and activity of receptor present. In adipose tissue, sympathetic stimulation causes lipolysis and in the liver it causes glycogenolysis. Both effects are mediated by β -adrenergic receptors. Thus, it is possible that metabolic changes caused by repeated restraint stress are receptor mediated events. Therefore, we determined β -adrenergic receptor binding activity in liver and adipose tissue by measuring the specific binding of the β -antagonist, ^3H dihydroalprenolol (DHA), to liver and adipose cell membrane from control, restraint and pair fed animals one day after the end of repeated restraint stress. There was no effect of stress on β -receptor number in the liver (data not shown) but the results from adipose tissue suggest a significant increase in the number of β -receptors present in tissue from restrained rats, compared with control or pair-fed animals (see Figure 3). Further studies are planned to confirm this observation.

We have previously found that the weight loss in repeatedly restrained rats is exaggerated by feeding the rats a high fat diet (Harris et al., 1998). As high fat diets induce obesity we hypothesized that the exaggeration of response was due to the adiposity of the high-fat fed rats and that weight loss in response to stress would be proportional to the size of body fat stores. Therefore, an experiment was conducted to determine whether stress-induced weight loss could be prevented if rats had lost a significant amount of weight prior to the onset of stress. Male Wistar rats were adapted to the 40% kcal fat diet and then divided into three groups: ad libitum, and two groups of food restricted rats. After the restricted rats had been fed 50% of their ad libitum food intake for 10 days half of each group was subjected to repeated restraint (3 hours of restraint on 3 consecutive days) and the other half was non-stressed controls. After the third day of restraint, the ad libitum group continued to eat ad libitum (AL) and one group of food restricted rats remained on a restricted food intake (FR) but the rats in the second food restricted group were returned to ad libitum feeding (FR-AL).

Body weights of the rats are shown in Figure 4. Restrained AL and FR-AL rats weighed significantly less than their respective non-stressed controls for all post-stress days. The restrained FR rats weighed significantly less than non-stressed FR rats for the first 5 days of recovery. Food intakes of the rats are also shown in Figure 4. Intakes of AL restrained rats were lower than those of their controls during stress. The intakes of all FR-AL groups was increased once food was freely available, but the restrained animals consumed less than their controls. The results from carcass analysis and serum hormones measures at the end of the experiment are shown in Table 1. Statistical analysis has not been completed on this data, however, it appears that repeated restraint caused a sustained reduction in body weight, even in animals that had already lost a significant amount of weight prior to stress. The results of this experiment demonstrate that weight loss induced by repeated exposure to an acute stress is not dependent upon the size of the body fat stores of rats prior to the onset of stress. We have demonstrated that this weight loss can be prevented using antagonists of hypothalamic CRF receptors during the period of restraint (Smagin et al., 1999) or by exposing the rats to restraint during the dark cycle instead of the light cycle (see below). Future experiments will determine the importance of circadian release of anabolic and catabolic hormones in mediating this response.

Table 1: Serum hormones and body composition of food restricted rats exposed to repeated restraint.

	AL		FR		FR-AL	
	<u>Control</u>	<u>Restrained</u>	<u>Control</u>	<u>Restrained</u>	<u>Control</u>	<u>Restrained</u>
<i>Day 12 Recovery</i>						
Corticosterone (ng/ml)	35 ± 10	57 ± 16	70 ± 16	153 ± 70	55 ± 12	117 ± 35
Insulin (ng/ml)	3 ± 0.2	3 ± 0.3	1 ± 0.1	2 ± 0.2	2 ± 0.2	2 ± 0.3
Leptin (ng/ml)	26 ± 2	19 ± 3	4 ± 0.5	4 ± 0.4	20 ± 2	14 ± 2
FFA (mEq/L)	0.6 ± 0.05	0.5 ± 0.05	0.5 ± 0.02	0.4 ± 0.04	0.4 ± 0.04	0.5 ± 0.03
Glucose (mg/dl)	269 ± 15	231 ± 9	216 ± 10	200 ± 13	260 ± 12	241 ± 15

Carcass Composition
(g/rat)

Weight	466 ± 16	434 ± 17	323 ± 7	314 ± 7	416 ± 13	391 ± 12
Protein	146 ± 5	140 ± 6	98 ± 3	95 ± 2	135 ± 6	112 ± 9
Water	260 ± 7	249 ± 7	201 ± 4	194 ± 7	240 ± 8	233 ± 9
Fat	47 ± 6	32 ± 6	10 ± 5	6 ± 1	29 ± 4	26 ± 2
Ash	14 ± 0.9	13 ± 0.4	13 ± 0.7	12 ± 0.6	13 ± 0.7	13 ± 0.4

Data are means ± sem for groups of 10 rats killed 12 days after the end of repeated restraint.

As stress activates a number of neural and hormonal systems that are not only involved in ingestion and metabolism, but also show circadian patterns of release, we have investigated how exposure to stress at different phases in the light/dark cycle affects ingestive behavior. We examined the effects of a single, 3 hour restraint stress, applied at the onset of the light (inactive) or dark (active) cycle, on ingestive behavior, body weight, and hormone levels. Food and water intake were measured during the subsequent light and dark cycles to determine the immediate and delayed effects of restraint. As shown in Figure 5, restraint during the light phase caused a significant reduction in body weight and food intake (Figure 6) during the subsequent dark cycle.

Restraint during the dark phase had no effect on body weight but the rats significantly increased their food intake during the subsequent light cycle. Corticosterone has a circadian pattern of release, with a peak at the onset of the dark cycle, therefore the proportional increase in corticosterone in response to stress was smaller in rats restrained during the dark phase but there were no differences in corticosterone measured the day after stress (see Table 2). These results demonstrate substantial diurnal differences in response to restraint stress. Some researchers have suggested that stress in the inactive phase would interfere with normal resting patterns and therefore be more aversive. Related to this is the possibility that stress has variable effects on metabolic rate and energy expenditure at different times during the diurnal cycle, which could effect both food intake and body weight. Further studies are needed to determine the role of circadian rhythms in the behavioral and physiological responses to stress.

Table 2: Stress and post-stress levels of serum corticosterone and leptin in rats exposed to 3 hours of restraint at the onset of the light or dark cycle.

	Corticosterone (ng/ml)		Leptin (ng/ml)	
	<i>Stress</i>	<i>Post stress</i>	<i>Stress</i>	<i>Post-stress</i>
LC	39 ± 10	232 ± 26	9.1 ± 1.3	8.7 ± 1.7
LS	411 ± 37 *	213 ± 13	7.0 ± 0.4	6.3 ± 1.3
DC	128 ± 24	257 ± 46	8.4 ± 1.7	6.9 ± 1.2
DS	284 ± 66 #	178 ± 24	6.1 ± 0.9	7.0 ± 1.1

Data are means ± sem for groups of 8 or 7 (DC) rats. Statistically significant differences were determined by two-way ANOVA. * significantly different from DS, P<0.05; ##

significantly different from controls, $P < 0.05$; ## significantly different from controls, $P < 0.0001$. Stress sampling occurred 1 hour into restraint for all groups; post-stress sampling occurred at the diurnal peak on Day 2, approx. 32h or 20h after stress for light and dark groups, respectively.

Very few studies have examined the effects of stress on macronutrient selection. Based on the differential effects of mild, severe, acute, and repeated stress on food intake, it would follow that macronutrient preference might also be affected by the type and intensity of the stressor. As there are several studies outlining the effects of mild stress on dietary selection, we chose to investigate how more severe stressors would affect the selection of diets high in carbohydrate, protein or fat. As described in a previous report, we used REM sleep deprivation as chronic stressor. We have now completed two experiments examining the effects of repeated restraint and of exercise stress in untrained rats on macronutrient selection.

In the first experiment male Sprague Dawley rats were divided into two weight-matched groups and fed either a control diet (10% kcal casein, 21 % kcal fat) or a three choice macronutrient diet: high carbohydrate (19% protein, 0.5% fat, 74% CHO), high protein (31% protein, 5% fat, 53% CHO), and high fat (19% protein, 23% fat, 22% CHO), ($n = 24$ per group). After 10 days each dietary group was further divided into two weight-matched groups (control and repeated restraint, $n = 12$ per group). Serum corticosterone was measured after the first hour of restraint on Day 1, as well as five days after the last day of restraint and upon sacrifice. In rats fed the selection diet, carbohydrate intake was decreased and protein intake was increased during stress (see Figure 7). Diet had no effect on corticosterone release during stress (data not shown) or the amount of weight lost during stress (Figure 7).

The data presented here shows that repeated restraint decreases the consumption of carbohydrates and increases the consumption of protein in a 3 diet macronutrient selection paradigm. The increased consumption of protein by restrained rats is particularly interesting as we have found that all of the weight loss experienced during the three days of stress is lean body mass, with no change in body fat content. This suggests that rats will increase protein intake if body energy stores are replete but lean mass has been lost. In the restrained rats larger changes in preference may have been observed if intake had been monitored at intervals during the day, as we have previously found that the period of greatest difference in intake of restrained and control rats is at the start of the dark period (Harris et al., 1998).

Repeated restraint is primarily a psychological stressor whereas the physical stress of treadmill running has been reported to acutely inhibit protein and carbohydrate intake (Larue-Achagiotis et al., 1993). Therefore, in the second study untrained rats were adapted to dry macronutrient selection diets (see Table 3) and forced to run on a treadmill for 60 minutes for each of three days to determine whether this stressor, which included a large physical component, had a significant effect on macronutrient selection. As with repeated restraint and sleep deprivation, running on the treadmill induced a significant weight loss which was not recovered during the 5 days after the end of stress (Figure 8). The rats consumed the largest portion of their energy intake as carbohydrate and approximately equivalent amounts of energy

as protein and fat (Figure 8). Energy consumed as each macronutrient before, during and after stress is illustrated in Figure 9. Stress inhibited energy intake and this was reflected as a decline in the amount of energy consumed from all three macronutrient diets. After stress protein intake rapidly returned to pre-stress levels but fat and carbohydrate intake remained lower than baseline intakes. The running stress caused a significant elevation of serum corticosterone (see Table 4). However, five days after the end of stress corticosterone was lower in the stressed rat than controls, this was also true for serum leptin and insulin.

Table 3: Diet Composition (g/100g) for Macronutrient Selection Study in Exercised Rats

	<u>Carbohydrate</u>	<u>Protein</u>	<u>Fat</u>
Cornstarch	54.5		
Sucrose	9.1		
Dextrin	27.2		
Casein		90.3	
DL methionine		1.4	
Shortening			77.5
Safflower oil			4.0
Vitamin mix	1.0	0.8	1.9
Mineral Mix	3.3	3.0	6.7
Choline Chloride	0.2	0.2	0.4
Fiber	4.7	4.3	9.5
Metabolizable energy (kcal/g)	3.69	3.35	7.44

Table 4: End point measures in rats exposed to treadmill running for an hour a day for three days.

	<u>Controls</u>	<u>Runners</u>
Day 2 Corticosterone (ng/ml)	20 ± 4	225 ± 22*
<i>Five Days Post-Stress</i>		
Leptin (ng/ml)	7.0 ± 0.6	4.6 ± 0.6*
Insulin (ng/ml)	2.5 ± 0.3	1.7 ± 0.3*
Corticosterone (ng/ml)	15 ± 5	5 ± 0.6*
Glucose (mg%)	92 ± 10	98 ± 5
FFA (uEq/L)	473 ± 85	439 ± 57
Carcass weight (g)	427 ± 8	415 ± 5
Carcass fat (%)	10.1 ± 0.6	9.5 ± 0.8

Data are means ± sem for 7 rats. Day 2 corticosterone was measured immediately after stress on the second day of stress. All other measures were made at the end of the study, five days after the end of stress. An asterisk indicates a significant difference between treatment groups ($P < 0.05$), determined by unpaired t-test.

In this experiment the use of a stressor that involved a significant physical component had a greater effect on energy intake of the rats than in the previous study in which the psychological stress of restraint was used. There are two factors that may contribute to the difference between the studies, as both the type of stress and the diet were different. Other

investigators using solid diets have reported that corticosterone stimulates fat intake (Castonguay 1991) but that central infusion of CRF inhibits fat intake of rats (David York, unpublished data). These observations, together with results from our two studies indicate that macronutrient selection in stressed animals is not determined by a single hormone, such as corticosterone, but is determined by multiple factors that are influenced by stress.

The Role Of Leptin In The Response To Repeated Restraint

Arica Guthrie, Tiffany Mitchell, Jacob Simpson, Ruth Harris

It has been reported that leptin modifies stress-responsiveness in mice. Ahima et al (1997) reported that a single injection of leptin inhibited corticosterone release in mice subjected to the physiological stress of starvation. In addition, Heiman et al (1997) have proposed that leptin may down regulate HPA activity to prevent maintained activation of the system following acute stress. In conditions of chronic stress, they suggest that leptin release would be down-regulated to allow the HPA axis to remain responsive. In a previous study we found a delayed decline in serum leptin concentrations of rats exposed to repeated restraint. Therefore, we identified a dose of leptin in rats that would produce a physiological response when infused continuously from an intraperitoneal Alzet pump.. Once this dose was identified we treated rats with leptin prior to exposure to repeated restraint in order to evaluate whether leptin inhibited any of the physiological responses associated with this stress.

In the pilot study male Sprague Dawley rats were infused with 50, 100 or 150 ug/day recombinant rat leptin (R&D Systems) for 7 days. The highest dose of leptin caused a transient inhibition of food intake during the first 2 days of leptin infusion but intake of this group returned to control levels by the end of the study (Figure 10). Weight change from baseline is shown in Figure 10. The rats receiving 50 ug/day and 150 ug/day leptin lost weight whereas control and those receiving 100 ug/day maintained baseline weight. There was a biphasic effect of leptin on glucose stimulated insulin release with the lowest dose of leptin inhibiting insulin release, implying increased insulin sensitivity. The high dose exaggerated insulin release, implying the development of insulin resistance (Figure 11). Carcass weight was consistent with the body weight measures in that there was a greater weight loss in rats receiving 50 and 150 ug/day leptin than in those infused with 0 or 100 ug/day leptin. Body fat showed a progressive increase with increasing doses of leptin, implying that higher doses of leptin promoted fat deposition but none of the differences were statistically significant (see Table 5).

Table 5: End point measures in rats infused with leptin

	0 ug/day	50 ug/day	100 ug/day	150 ug/day
Pre-infusion wt. (g)	390 \pm 5	398 \pm 4	394 \pm 4	391 \pm 4
Carcass Wt (g)	362 \pm 4	356 \pm 3	358 \pm 3	355 \pm 4
Carcass Fat (%)	7.8 \pm 0.7	8.1 \pm 0.7	8.4 \pm 0.9	8.8 \pm 0.6
<i>Serum Hormones</i>				
Leptin (ng/ml)	5.1 \pm 0.5	6.5 \pm 0.7	7.4 \pm 0.5	8.1 \pm 0.7
Corticosterone (ng/ml)	11 \pm 22	9 \pm 1	11 \pm 1	14 \pm 4

Data are means \pm sem for groups of 8 rats killed after 7 days of leptin infusion.

As the pilot study indicated that low and high doses of leptin were likely to have different effects, we used two doses of leptin in the next experiment which was designed to determine whether leptin would influence the response to repeated restraint. Male Sprague Dawley rats were fed high fat diet (40% kcal fat) for ten days and then infused with 0, 30 or 100 ug/day leptin for 28 days. There was no effect of leptin on insulin response to a glucose challenge, or on glucose response to an insulin challenge (data not shown). There were no differences in food intakes or body weights of the rats which were exposed to repeated restraint after 21 days of leptin infusion (see Figure 12) although the infusion caused significant increases in serum leptin concentrations (Control: 4.7 ± 0.6 , 30 ug/d: 6.3 ± 0.5 , 100 ug/d: 16.5 ± 2.6 ng/ml). Restraint caused significant reductions in body weights and food intakes of all three treatment groups (see Figure 13) and the amount of weight lost was similar for all three treatments (~14 g over 3 days).

Figure 14 shows serum corticosterone and leptin measured during and after stress. Corticosterone was elevated in all rats during the restraint period. The blood sample was collected after 1 hour of restraint on the first day of restraint and it is possible that the disruption of moving the controls to the same room as restrained rats was stressful. The 30 ug/day leptin group seemed to have a slightly increased HPA response to stress compared with controls. The day after stress there were no differences in corticosterone. Serum leptin appeared to be elevated one day after stress and showed significant elevations in the rats infused with 100 ug/day. All of the rats had higher serum leptin concentrations by the end of the experiment than had been measured 2 days after pump placement (see above). These changes in leptin may be explained in part by the body fat content of the rats as the control rats infused with leptin had more fat than the PBS infused controls (see Table 6). However, it is also possible that the 100 ug/day rats did not clear leptin from the blood as fast as it was being infused, resulting in a gradual elevation in serum leptin concentration over the course of the study. Body composition measured at the end of the study is shown in Table 6. All of the restrained rats lost similar amounts of weight compared with their non-stressed controls, however, the composition of loss was different between groups. PBS infused restrained rats lost small amounts of body fat and protein but the majority of weight loss was accounted for by water. In rats infused with leptin a large portion of the weight loss was accounted for by fat and the high dose of leptin did not prevent loss of protein but did retain carcass water. Thymus weight was reduced in all restrained rats but reduction in thymus size was greater in rats receiving leptin than in the PBS controls. The thymus is the primary site of T cell production, suggesting that leptin may exaggerate the effects of stress on immune function.

Table 6: Body composition of rats infused with leptin and exposed to repeated restraint stress

	0 ug/day		30 ug/day		100 ug/day	
	Control	Restrained	Control	Restrained	Control	Restrained
Carcass Wt (g)	409 ± 6	393 ± 9	404 ± 8	388 ± 9	408 ± 7	396 ± 9
Fat (g)	45 ± 4	43 ± 4	46 ± 3	40 ± 4	50 ± 1	39 ± 3
Protein (g)	96 ± 1	94 ± 2	94 ± 3	93 ± 3	99 ± 2	92 ± 4

Water (g)	252 \pm 3	243 \pm 5	251 \pm 4	241 \pm 5	248 \pm 4	252 \pm 3
Thymus wt (mg)	374 \pm 35	330 \pm 26	357 \pm 30	291 \pm 24	375 \pm 30	279 \pm 19

Data are means \pm sem for groups of 8 rats killed 5 days after the end of repeated restraint. Stress caused weight loss in all groups but in the rats given 100 ug/day leptin the majority of the loss was body fat whereas it was lean tissue in rats receiving 0 ug/day leptin. Leptin exaggerated the effects of stress on thymus weight.

The Effects of Rapid Eye Movement Sleep Rebound on Spatial Learning and Physiology of Rats

Bradley D. Youngblood and Ruth B. S. Harris

In previous experiments, we have shown that 48 to 96 hours of rapid eye movement sleep deprivation (REMSD) by the flower pot technique impairs spatial learning in rats tested in a Morris Water Maze (Youngblood et al., 1997). Sleep deprivation also disrupts physiological systems, causing an increase in rectal temperature and progressive weight loss. We carried out two experiments to determine whether the spatial reference memory and physiological deficits were permanent or transient in response to 96 hours REMSD. In the first study male Wistar rats were divided into two groups, one was controls and the other was subjected to 96 hours of REMSD by the flower-pot method (Youngblood et al., 1997). Spatial memory was tested in a Morris Water Maze Place Learning Set Task (Youngblood et al., 1997) after 96 hours of sleep deprivation and after 12 and 24 hours of rebound sleep. Sleep deprivation caused a decrement in working memory, measured in trial 1 of the Place Learning set task, and this decrement was still apparent after 12 hours of rebound sleep but not after 24 hours. Sleep deprivation caused a significant weight loss, which was not recovered, and an elevation of body temperature but the REMSD rats were hypothermic after 24 hours of rebound sleep.

In the second study rats were sleep deprived for 96 hours and then followed during 5 days of rebound sleep. REMSD had no effect on food intake but caused a significant weight loss which was not recovered by the end of the experiment (see Figure 15). The REMSD rats were hyperthermic during sleep deprivation, but hypothermic after 12 hours of rebound sleep (see Figure 16), confirming results from the previous experiment. Serum corticosterone was elevated during sleep deprivation, but was normal during the recovery period. In contrast thymus weight was decreased in REMSD rats, even after 5 days of rebound sleep (see Figure 17). In previous studies with REMSD rats we have found a significant increase in serotonin turnover in various brain areas after 96 hours of sleep deprivation (Youngblood et al., 1997). In this study serotonin metabolism, expressed as a ratio of 5-HIAA to 5-HT, was elevated in the hippocampus, hypothalamus and brain stem at the end of sleep deprivation but was lower in the hippocampus from REMSD rats than control rats during the recovery period (see Figure 18).

The results from these experiments demonstrate that recovery from a period of sleep deprivation is associated with a number of physiologic and cognitive responses that include a drop in body temperature and a decrease in hippocampal serotonin metabolism that is associated

with improved performance in a spatial learning task. The specificity of this change to the hippocampus, as it was not apparent in the hypothalamus or brain stem, support our hypothesis that the decrements in spatial memory of chronically stressed rats is associated with changes in serotonin metabolism in the hippocampus, an area of the brain that is known to integrate neural activity that controls memory. In contrast to the reversal of these responses, the increased swim speed of REMSD rats was not corrected, suggesting mediation by a pathway that is chronically disrupted by stress. In addition, loss of weight induced by sleep deprivation is not recovered during the period immediately after stress, similar to the response observed in rats exposed to repeated restraint. We have hypothesized that this permanent down-regulation of body weight may be associated with a stress-induced disruption of circadian rhythms and the unexpected drop in body temperature of the REMSD rats following rebound sleep would be consistent with this hypothesis.

Involvement of Urocortin in the Response to Stress

Gennady Smagin, Xiaolang Yan, Leigh Anne Howell and Mingxia Shi

Urocortin (UCN), a mammalian peptide with some homology to CRF and a high affinity for CRF receptors, has been implicated in endocrine and behavioral responses to stress. However, the physiological role of UCN is not clear as infusion studies suggest that urocortin and CRF are capable of mediating similar responses but under normal conditions UCN may not be present in the areas that mediate the end point response, therefore, we have examined mechanisms regulating UCN mRNA expression during stress. UCN messenger RNA has been detected in the hippocampus, Edinger-Westphal nucleus, hypothalamus, substantia nigra, cerebellum and pituitary gland (Wong, al-Shekhlee et al. 1996). UCN-like immunoreactivity has been found in the hypothalamus (supraoptic, paraventricular and ventromedial nuclei), raphe nuclei and substantia nigra (Kozicz et al. 1998). The greatest density of cells were found in the Edinger-Westphal nucleus.

To quantify UCN gene expression in response to various stimuli, we used an RNase protection assay (RPA). Hybridized mRNA fragments were detected using a Phosphorimaging system. Levels of rat β -actin mRNA were quantified simultaneously to normalize the amount of total RNA used for each sample. As shown in Figure 19, 1 hour of restraint stress significantly increased UCN mRNA levels in the hypothalamus and midbrain (a region that included the Edinger-Westphal nucleus). At the same time, CRF mRNA was not affected in either brain region. It has been shown previously that mature CRF mRNA levels increase in the hypothalamus 90-120 min after the onset of stress, whereas heteronuclear CRF RNA (hnRNA) is significantly increased 10-30 minutes after onset of stress (Imaki et al. 1995). Our probe detects mature CRF mRNA. As shown in Figure 20, in the hypothalamus of adrenalectomized (ADX) animals the levels of UCN mRNA were not significantly changed compared with the control group, while the levels of CRF mRNA were significantly increased by 96 hours. A different pattern of activation was observed in the midbrain (Figure 20) in which UCN mRNA levels were significantly elevated 24 hours after ADX, and return to the level of sham-controls 96 hours after surgery. At the same time, CRF mRNA levels in the midbrain were not affected by ADX

(Figure 20). The data are in agreement with previous reports on regulation of hypothalamic CRF mRNA by adrenal steroids and demonstrate that UCN gene expression is inducible by ADX and is possibly regulated by adrenal steroids in a site specific manner. When ADX rats received corticosterone (CORT) replacement (35 ug/ml in drinking water), CORT replacement (ADX+CORT) significantly attenuated the ADX-induced increase in UCN and CRF mRNA levels (Figure 21).

The first system to be activated by stress is the brain stem catecholaminergic system, therefore, we determined whether the level of UCN mRNA was affected by administration of a noradrenergic agonist, norepinephrine (NE). Rats were implanted with the ICV cannula into the 3rd cerebral ventricle and adrenalectomized. 200 nM of NE in 2 µl or 2 µl of sterile saline were injected through the cannula. As shown in Figure 22, 60 minutes after the injection NE increased hypothalamic UCN and CRF mRNA but UCN mRNA in the midbrain was not significantly affected. Activation of the CRF system after ICV administration of NE agrees with previously published data of Itoe et al. (1994).

The results of these studies demonstrate that endocrine factors regulate UCN expression and may be responsible for stress-induced stimulation of UCN mRNA expression. In additional studies, we discovered a naturally occurring antisense UCN transcript, using an RNase protection assay. Natural antisense RNAs are endogenous transcripts that exhibit complementary sequences to known mRNAs. There is increasing evidence that antisense RNAs regulate sense RNA expression. As shown in Figure 23, we have found tissue-specific expression of antisense UCN that is increased by 1 hour of restraint stress (Figure 24). Our current objective is to characterize this antisense transcript by molecular cloning. Identification of antisense UCN RNA will provide valuable information on how UCN is regulated. Further studies are needed to determine which aspects of the immune, endocrine, physiological and behavioral responses are mediated by UCN and which are secondary to CRF release. These studies will be difficult to complete until specific agonists and antagonists are available as both CRF and UCN are stimulated by stress and they activate the same receptors.

The Effect of Restraint Stress on Brain Glucose and Palmitate Utilization

Ruth Harris, Bradley Youngblood, Jun Zhou, Gennady Smagin

There is a significant amount of evidence that glucose or glucose analogue supplementation in animals improves memory and acts as an analgesic, raising the threshold for pain (Reddy et al., 1998). Studies with human subjects, performed by Natick Laboratories, have indicated an improvement in physical performance and vigilance of Rangers when they consume carbohydrate during stressful exercise. The objective of these preliminary studies was to determine whether we could detect any effect of stress on brain energy utilization, with the intention of using the model to identify the optimal composition and timing of glucose supplements for improvement of mental and physical performance.

The results of two pilot studies are shown in Figure 25. In the first study we measured glucose and fatty acid utilization in five brain areas of rats that were either non-stressed controls or had been restrained for 3 hours (n=6). Although there were no significant effects of restraint on glucose or fatty acid utilization, there were obvious trends that would become significant with a larger number of animals per group. Restraint had no effect on glucose oxidation but decreased glucose incorporation into fatty acids in the LH and VMH. Stress increased fatty acid oxidation in the hippocampus and increased fatty acid esterification in the LH, VMH, hippocampus and cerebellum. In the second study we found no effect of 1 hour of restraint on the same measures (data not shown).

These preliminary results suggest that 3 hours of restraint influences nutrient metabolism in specific areas of the brain which have been implicated in the regulation of energy balance and in cognitive function (Kasser et al., 1985). More work is needed to determine the neurotransmitter pathways that initiate the response and the implications for physical and cognitive behavior. Future experiments will measure glucose and fatty acid utilization in a rat model that provides a better parallel of the human studies conducted by Natick. Rats will be trained on a treadmill for two weeks and will then be subjected to an exhaustive bout of exercise. Glucose utilization in both the brain and peripheral tissues will be determined.

Genetic Markers for Stress Responsiveness:

The Effect of Restraint on Mice Overexpressing Agouti Protein

Ruth Harris, Tiffany Mitchell, Jacob Simpson, Jun Zhou and Mingxia Shi

We have conducted studies to identify peripheral markers of stress responsiveness. Previously we have reported that mice depleted of Apolipoprotein E have an increased sensitivity to stress (Zhou et al., 1998). We are currently investigating agouti protein which is an antagonist of melanocortin receptors (MC-R). MC1-R is the receptor for melanocyte-stimulating hormone (MSH) which determines skin and fur pigmentation, therefore, mice overexpressing agouti have a yellow coat color. Agouti also antagonizes MC4-R which has been implicated in the regulation of food intake. Agouti mice and mice in which MC4-R has been knocked out are hyperphagic and moderately obese (Fan et al, 1997; Huszar et al., 1997). MC2-R, or the adrenocorticotrophic receptor, may also be antagonized by agouti (Tatro, 1996), therefore, it seemed likely that agouti mice would show an altered sensitivity towards stress if the HPA axis is disrupted.

We have conducted two studies using wild type and transgenic (BAP) mice in which agouti was expressed ectopically by linking it to a -actin promoter. This resulted in a yellow coat color and mild obesity (Klebig et al., 1995). In the first study the animals were exposed to our repeated restraint procedure, which has been shown to cause sustained weight loss in rats (Harris et al., 1998). BAP mice exposed to 2 hours of restraint on each of 3 days lost more weight than wild type mice exposed to the same stress (see Figure 26). The non-restrained BAP mice also lost weight, suggesting that the manipulations involved in the experiment were more stressful to the BAP mice than the wild type animals. Serum measures of corticosterone are in

progress to determine whether the differences between BAP and wild type mice are associated with differences in HPA activation during stress. It is anticipated that agouti will have blocked ACTH activity in BAP mice.

In a second study BAP mice and wild type C57BL6J mice were subjected to 12 minutes of restraint and then anxiety-type behavior was measured in a defensive withdrawal paradigm. The defensive withdrawal apparatus consisted of a brightly lit 0.5m square open field with a white floor. The open field was divided into a central and a peripheral zone. A cylindrical black chamber (length 10 cm, diameter 6.5 cm), open at one end, was secured to the floor lengthwise next to one wall and 20 cm away from the corner. To start the test, a mouse was placed in the chamber and behaviors were observed during a 5 min session using a Video Tracking, Motion Analysis & Behavior Recognition System (Noldus Information Technology, Wageningen, the Netherlands Sterling VA, U.S.A. As shown in Table 6, the BAP mice show more anxiety-type behaviors (reduced entries and less time spent in the center zone) and they move more quickly than the wild type mice. Since these differences were observed in both control and restraint BAP mice, they are related to genotype rather than stress responses. Also, since the control BAP mice already displayed stress-type behaviors, compared with wild type mice, restraint did not further change the behavior in BAP mice.

The results of these two studies suggest that BAP mice have an increased sensitivity to restraint stress. However, the data presented here is only a preliminary and more animals are needed to confirm the results. In addition, the mice need to be tested in different behavioral paradigms, such as a light-dark box test, to confirm the results obtained in the defensive withdrawal test. Once we have established that agouti protein increases sensitivity towards stress, we will use various mouse models to determine which aspect of the melanocortin system is responsible for the sensitization.

Table 6. Comparison of defensive withdrawal behavior of BAP and wild type mice in response to restraint stress.

Measurements	<i>Wild type mice</i>		<i>BAP mice</i>	
	Control	Restraint	Control	Restraint
Distance moved (cm)	3337 ± 299 ^A	3053 ± 358 ^A	4589 ± 470 ^B	4391 ± 975 ^B
Velocity (cm/s)	11.4 ± 1.0 ^A	10.7 ± 1.2 ^A	16.5 ± 1.5 ^B	15.4 ± 3.4 ^B
Number of Rears	25.0 ± 2.9	19.5 ± 2.7	23.9 ± 6.6	21.6 ± 5.2
Time spent rearing (sec)	19.0 ± 2.9	15.0 ± 2.3	21.5 ± 6.4	27.0 ± 6.4
Grooming number	2.5 ± 0.5	18.7 ± 5.3 ^A	0.7 ± 0.3	1.3 ± 0.9
Grooming time (sec)	4.5 ± 0.8	33.5 ± 10.6 ^B	2.0 ± 0.9	5.3 ± 3.7
Number of exits from chamber	3.9 ± 0.4	3.1 ± 0.6	2.4 ± 0.6	2.6 ± 0.8

Time out of chamber (sec)	266.4 ± 4.0	249.8 ± 21.1	195.1 ± 39.7	217.4 ± 23.0
Entries into center zone	24.5 ± 6.9 ^A	16.0 ± 2.5 ^A	13.1 ± 4.2 ^C	9.0 ± 1.5 ^C
Center zone time (sec)	30.7 ± 3.6 ^A	19.6 ± 3.9 ^A	15.4 ± 5.1 ^B	16.6 ± 3.8 ^B

Data are means ± SE for groups of 10 wild type and 7 agouti mice. The different letters represent significant differences, determined by two way ANOVA.

Reportable Outcomes

Jun Zhou completed requirements for her Ph.D. in Veterinary Sciences at Louisiana State University. Her graduate research project was supported by this grant and her thesis title is "The effect of repeated restraint stress on peripheral energy utilization in rats". Her diploma will be awarded in August, 1999.

You Zhou, Ph.D. left the group in July 1998 to take a Research Assistant Professor position at University of Nebraska Lincoln. His responsibilities include Manager of the Microscopy Core Research Facility and Director of the Antibody Core Facility.

Gennady Smagin, Ph.D. is leaving the group this month to take a faculty position at Louisiana State University Medical Center in Shreveport. This is a joint appointment at the Departments of Psychiatry and Pharmacology,

Arica Guthrie, a high school student from Washington, DC, spent 8 weeks in the laboratory during the summer of 1998 as a participant in the NASA SHARP PLUS program sponsored through Southern University, Baton Rouge. She conducted the leptin dose response study that is described in the body of this report.

Manuscripts

Smagin, G.N., L.A. Howell, D.H. Ryan, E.B. DeSouza, R.B.S. Harris. Corticotrophin releasing factor (CRF)- and urocortin (UCN)-induced anorexia in rats: the role of CRF₂ receptors. *NeuroReport* 9: 1601-1606, 1998

Howell, L.A., R.B.S.Harris, C. Clarke, B.D. Youngblood, D.H. Ryan and T.G. Gilbertson. The effects of acute or repeated restraint stress on taste preferences in rodents. *Physiol. Behav.* 65: 697-704, 1999.

Harris, R.B.S., J. Zhou, B.D. Youngblood, I.I. Rybkin, G.N. Smagin, D.H. Ryan. The effect of repeated restraint on body weight and composition of rats fed low and high fat diets. *Am. J. Physiol.* 275: R1928-R1938, 1998.

Zhou, J., X. Yan, D.H. Ryan, R.B.S. Harris. Sustained effects of repeated restraint stress on muscle and adipocyte metabolism in high fat fed rats. *Am. J. Physiol.* In Press

Zhou, Y., A. Cheshire, L.A. Howell, D.H. Ryan, R.B.S. Harris. Neuroautoantibody immunoreactivity in relation to aging and stress in apolipoprotein E-deficient mice. *Brain Res.* In Press.

Smagin, G.N., L.A. Howell, S. Redmann, D.H. Ryan, R.B.S. Harris. Prevention of stress-induced weight loss by third ventricle corticotropin releasing factor receptor antagonist. *Am. J. Physiol.* 276: R1461-R1468, 1999.

Smagin, G.N., L.A. Howell, D.H. Ryan, R.B.S. Harris. Central administration of urocortin activates cerebral monoaminergic systems in rats. *Neuroscience Res.* Submitted

Smagin, G.N., X. Yan, D.H. Ryan, R.B.S. Harris. Urocortin messenger RNA levels in the hypothalamus and midbrain are modulated by restraint stress and glucocorticoids. *NeuroReports.* Submitted.

Youngblood, B.D., G.N. Smagin, P.D. Elkins, D.H. Ryan, R.B.S. Harris. Sleep deprivation and valine effect spatial learning and brain 5-HT metabolism. *Physiol. Behav.* In Press

Book Chapters

Harris, R.B.S., L.A. Howell, T. Mitchell, B.D. Youngblood, D.A. York and D.H. Ryan. Stress and macronutrient selection. In: *Neural control of macronutrient selection.* CRC Press (in press) 2000.

Abstracts

Youngblood, B.D., G.N. Smagin, D.H. Ryan, R.B.S. Harris. Dietary histidines effect on regional brain histamine and spatial learning in continuously stressed rats. *Soc. Neurosci. Abstr.* 24: Abst 371.14, 1998

Zhou, Y., L.A. Howell, D.H. Ryan, R.B.S. Harris. Neuroautoantibody reactivity in relation to aging and stress in apolipoprotein E deficient mice. *Soc. Neurosci. Abstr.* 24: Abst 763.6, 1998

Yan, X., Y. Zhou, G.N. Smagin, I. Kim, D.H. Ryan, R.B.S. Harris. A natural urocortin antisense RNA is detected by RNase protection assay in heart and hypothalamus of the rat. *Soc. Neurosci. Abstr.* 24: Abst 546.8, 1998

Smagin, G.N., D.H. Ryan, E.B. DeSouza, R.B.S. Harris. The role of CRF₁ receptors in restraint-, CRF- and UCN-induced anxiety-like behaviors. *Soc. Neurosci. Abstr.* 24: Abst 471.15, 1998

Howell, L.A., G.N. Smagin, D.H. Ryan and R.B.S. Harris. CRF antagonist helical CRF⁹⁻⁴¹ (hCRF) attenuates the effects of repeated restraint stress on food intake and body weight independent of acute HPA axis activation. Soc. Neurosci. Abstr. 24: Abst 469.10, 1998

Zhou, J, Yan, D.H. Ryan and R.B.S. Harris. Post stress effects on glucose and fatty acid metabolism in rats. FASEB J. Abst 201.8, 1999

Zhou, Y., L.A. Howell, G.N. Smagin. D.H. Ryan and G.N. Smagin. Study of central regulation of stress responses in Apolipoprotein E-deficient mice. Neuroscience, 1999.

Mitchell, T.M., J. Simpson and R.B.S. Harris. Leptin does not prevent stress induced weight loss in rats. Neuroscience, 1999.

Youngblood, B.D., D.H. Ryan and R.B.S. Harris. The effects of sleep deprivation and sleep rebound on spatial learning and brain serotonin metabolism in rats. Neuroscience 1999.

Smagin, G.N., X. Yan, D.H. Ryan and R.B.S. Harris. Urocortin mRNA levels in the hypothalamus and midbrain are modulated by restraint stress and glucocorticoids. Neuroscience 1999.

Key Research Accomplishments

- Publication of nine manuscripts in peer reviewed journals.
- Ten poster presentations at international meetings
- Identification of naturally occurring urocortin antisense mRNA
- Demonstration of regulation of urocortin expression by adrenal hormones.
- Identification chronic changes in peripheral energy metabolism following acute exposure to stress.

C. Conclusions

The results from our studies investigating behavior and physiology in rats during the recovery period following acute or chronic stress provides some of the first evidence that there are long-lasting effect of stress on cognitive and metabolic functions. Understanding these responses will facilitate development of protocols for promoting rapid recovery from stressful conditions. Identification of factors influencing UCN expression provides evidence for a role for UCN in the stress response and further studies are needed to clarify which biological responses to stress, all of which previously have been attributed to CRF, may be mediated by UCN. The preliminary studies examining the effects of stress on brain glucose metabolism will be changed

to parallel human studies carried out at Natick in order to provide an animal model for testing the optimal time and composition of nutritional supplements that improve physical and cognitive function during extreme physical stress. Continuing investigation of proteins that act as peripheral markers for stress responsiveness may ultimately lead to a routine screen that will predict the potential for cognitive or physical performance of an individual in stressful environments.

D. References

1. Ahima, R. S. Prabakaran, D. Mantzoros, C. Qu, D. Lowell, B. Maratos-Flier, E. Flier, J. S. Role of leptin in the neuroendocrine response to fasting *Nature* 382: 250-252, 1996.
2. Castonguay T.W. Glucocorticoids as modulators in the control of feeding. *Brain Research Bulletin* 27: 423-428, 1991.
3. Fan, W., B. A. Boston, R. A. Kesterson, V. J. Hruby, and R. D. Cone. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165-168, 1997.
4. Germack, R., A. B. Starzec, R. Vassy, and G. Y. Perret. Beta-adrenoceptor subtype expression and function in rat white adipocytes. *British Journal of Pharmacology* 120: 201-210, 1997.
5. Harris, R. B. S., J. Zhou, B. D. Youngblood, I.I. Rybkin, G. N. Smagin, D. H. Ryan. The effect of repeated restraint stress on body weight and body composition of rats fed low and high fat diets *American Journal of Physiology* 275: R1928-1938, 1998.
6. Heiman, M. L. Ahima, R. S. Craft, L. S. Schoner, B. Stephens, T. W. Flier, J. S. Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* 137: 3859-3863.
7. Huszar, D., C. A. Lynch, V. Fairchild-Huntress, J. H. Dunmore, Q. Fang, L. R. Berkemeier, W. Gu, R. A. Kesterson, B. A. Boston, R. D. Cone, F. J. Smith, L. A. Campfield, P. Burn, and F. Lee. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88: 131-141, 1997.
8. Imaki, T., W. Xiao-Quan, et al. Stress-induced activation of neuronal activity and corticotropin-releasing factor gene expression in the paraventricular nucleus is modulated by glucocorticoids in rats. *Journal of Clinical Investigation* 96: 231-238, 1995.

9. Itoi, K., A. F. Seasholtz, S.J. Watson. Cellular and extracellular regulatory mechanisms of hypothalamic corticotropin-releasing hormone neurons. *Endocrine Journal* 45: 13-33 1998.
10. Itoi, K., T. Suda, F. Tozawa, I. Dobashi, N. Ohmori, Y. Sakai, K. Abe, H. Demura. Microinjection of norepinephrine into the paraventricular nucleus of the hypothalamus stimulates corticotropin-releasing factor gene expression in conscious rats. *Endocrinology* 135: 2177-2182, 1994.
11. Kozicz, T., H. Yanaihara, A. Arimura. Distribution of urocortin-like immunoreactivity in the central nervous system of the rat. *The Journal of Comparative Neurology* 391: 1-10, 1998.
12. Kasser, T. R., R. B. Harris, and R. J. Martin. Level of satiety: fatty acid and glucose metabolism in three brain sites associated with feeding. *American Journal of Physiology* 248: R447-452, 1985.
13. Klebig, M. L., J. E. Wilkinson, J. G. Geisler, and R. P. Woychik. Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur. *Proceedings of the National Academy of Sciences of the United States of America* 92: 4728-4732, 1995.
14. Larue-Achagiotis, C. Martin, C. Verger, P. Chabert, M. Louis-Sylvestre, J. Effects of acute treadmill exercise and delayed access to food on food selection in rats. *Physiology and Behavior* 53: 403-408, 1993.
15. Reddy, P. R., R. S. Raman, and S. Ramaswamy. Effect of glucose on stress induced antinociception in normal mice. *Indian Journal of Physiology & Pharmacology* 42: 131-134, 1998.
16. Rybkin, I. Y. Zhou, J. Volaufova, G. N. Smagin, D. H. Ryan, and R. B. S. Harris. Effect of restraint stress on food intake and body weight is determined by time of day. *American Journal of Physiology* 273: R1612-R1622, 1997.
17. Smagin, G.N., L.A. Howell, S. Redmann, D.H. Ryan, R.B.S. Harris. Prevention of stress-induced weight loss by third ventricle CRF receptor antagonist *American Journal of Physiology* 276, 1461-1468, 1999
18. Tatro, J. B. Receptor biology of the melanocortins, a family of neuroimmunomodulatory peptides. *Neuroimmunomodulation* 3: 259-284, 1996.

19. Wong, M. L., A. al-Shekhlee, P.B. Bongiorno, A. Esposito, P. Khatri, E.M. Sternberg, P.W. Gold, J. Licinio. Localization of urocortin messenger RNA in rat brain and pituitary. *Molecular Psychiatry* 1: 307-312, 1996.
20. Youngblood, B. D. Zhou, J. Smagin, G. N. Ryan, D. H. Harris, R. B. Sleep deprivation by the "flower pot" technique and spatial reference memory. *Physiology and Behavior* 61: 249-256, 1997
21. Zhou, J., X. Yan, D.H. Ryan, R.B.S. Harris. Sustained Effects of Repeated Restraint Stress on Muscle and Adipocyte Metabolism in High Fat Fed Rats *American Journal of Physiology* In Press, 1999.
22. Zhou, Y., P. D. Elkins, L. A. Howell, D. H. Ryan, and R. B. Harris. Apolipoprotein-E deficiency results in an altered stress responsiveness in addition to an impaired spatial memory in young mice. *Brain Research* 788: 151-159, 1998.

IV. Stress, Nutrition and Work Performance

A. Introduction

Nutritional needs of soldiers have been evaluated on a periodic basis since World War II, with responsibility for prescribing nutritional standards for operational (deployment) rations belonging to the Army Surgeon General. Nutrient content of operational rations, to include protein, has been determined by reviewing published research and incorporating the judgment of experts; emphasis is on optimal requirements rather than minimum daily requirements. After reviewing research on energy expenditure and nutrient consumption of soldiers during World War II, the Army established requirements of 3600 kcal and 100 g protein per day for physically active personnel deployed in temperate climates (AR 40-250, 1947). Additional requirements for environmental extremes were not addressed, and no changes have been made in those requirements since they were initially established.

Soldiers, when deployed or on field training exercises, are expected to perform physical activity at a level that may be well above their normal activity level while in garrison. Although energy expenditure may be increased, energy intake from foods consumed in the field is less than energy intake in garrison, ranging from 2009-3050 kcal in the field versus 2773-3173 kcal in garrison for men (Baker-Fulco, 1995; Thomas et al., 1995; Cline et al., 1997; Tharion et al., in press); and ranging from 1658-2343 kcal in the field versus 1832-2592 kcal in garrison for women (Edwards et al., 1991; Rose et al., 1989; Hirsch et al., in press; King et al., 1994; Cline et al., 1996).

Total daily energy expenditure has been measured in male soldiers during strenuous field training exercises, ranging from approximately 3400 kcal to over 5000 kcal in various settings, and energy deficits of 520 kcal to almost 2000 kcal per day have been measured when comparing

energy expenditure with energy intake during field operations (Tharion et al., 1997; Baker-Fulco, 1995; Shippee et al., 1994).

Protein requirements were established in 1947 for a reference male soldier of 68 kg, with 100 g providing 1.47 g/kg. The current reference weight of soldiers is 78 kg, which would translate to 1.28g/kg of protein. Recent studies on consumption patterns of military personnel have reported an intake of 1.34-1.64 g/kg of protein in garrison, and an intake of 1.14-1.65 g/kg in field operations (Cline et al., 1997; Thomas et al., 1995; Rose and Carlson, 1986; Askew et al., 1986; Morgan et al., 1988; Edwards et al., 1991).

It is known that regular exercise increases protein needs, with intakes of 12-15% of energy from protein recommended, unless energy intake is insufficient. Insufficient energy intake can lead to negative nitrogen balance, even at protein intakes that have promoted positive nitrogen balance at adequate energy intakes (Munro, 1951; Butterfield and Calloway, 1984; Calloway and Spector, 1954; Goran and Forsum, 1985; Walberg et al., 1988). Current evidence suggests that strength or speed athletes should consume about 1.2-1.7 g/kg of protein and endurance athletes about 1.2-1.4 g/kg (Lemon, 1991). Inadequate carbohydrate (CHO) intake will cause more rapid depletion of muscle and liver glycogen during exercise and may contribute to greater protein utilization (Anderson and Sharp, 1990; Lemon and Mullin, 1980; MacLean et al., 1989). Because of the established link between CHO intake and optimal performance in high-demand exercise (Costill, 1988), military requirements for daily CHO intake have been set at a minimum value 440 g, or 48.9 percent of energy. Current studies on military field operations have reported inadequate mean CHO intake, ranging from 244-369 g; energy deficits were also reported for the populations studied (Askew et al., 1986; Morgan et al., 1988; Edwards et al., 1991).

Because soldiers are self selecting a diet deficient in energy and carbohydrate but maintain protein intake when deployed or on field training exercises, it is of interest to know whether: 1) the self-selected protein content is adequate to maintain nitrogen balance, and 2) whether an energy (and CHO) deficient diet impairs physical performance. Therefore, the specific objectives of this project were: A) to determine the effects of moderate (750 kcal/day) caloric restriction in combination with daily exercise (500 kcal energy expenditure) on body composition, muscular strength, aerobic (endurance) performance, and anaerobic capacity, and B) to determine the effects of moderate (750 kcal/day) caloric restriction in combination with daily exercise (500 kcal energy expenditure) on protein balance as determined from urinary nitrogen excretion.

B. Body

Subjects

Thirteen men and 11 women who regularly performed aerobic exercise were recruited to participate in this study. All were healthy as determined by medical history and physical

examination, which included routine blood and urine chemistries. None had experienced significant weight loss/gain (>5 kg) in the previous 6 months, nor were any of the volunteers routinely consuming dietary supplements such as creatine monohydrate. All had a habitual dietary protein intake of between 0.8 and 2.0 g/kg body weight/day. Physical characteristics of the subjects are given in Table 1. The Pennington Center and Department of Defense Institutional Review Boards approved this study and all subjects gave written informed consent.

Table 1: Physical Characteristics of Study Participants (Data are mean \pm SE)

	Age (yrs)	Weight (kg)	% Body Fat (DEXA)	BMI	VO ₂ max (ml/kg/min)
Total (n=24)	23.6 \pm 0.9	71.7 \pm 2.4	22.9 \pm 1.6	24.1 \pm 0.6	51.1 \pm 1.0
Men (n=13)	25.5 \pm 1.2	80.1 \pm 2.4	19.3 \pm 2.0	25.3 \pm 0.8	53.4 \pm 1.4
Women (n=11)	21.4 \pm 0.9	61.8 \pm 2.0	27.6 \pm 1.9	22.7 \pm 0.7	48.8 \pm 1.3

Experimental Design

This study was designed to address whether moderate energy restriction (750 kcal/day) in combination with daily exercise (500 kcal energy expenditure) would have detrimental effects on muscular strength, endurance, and anaerobic performance in exercise-trained men and women. Prior to experimental testing, basal metabolic rate was determined by indirect calorimetry and maximal oxygen consumption (VO₂) was measured in all subjects during an incremental test to exhaustion while running on a treadmill. Before the start of the study, subjects completed a three day food record which was used to determine habitual energy and protein intake. In order to (1) decrease any potential experimental artifact related to individual differences in energy balance and diet composition during the baseline period (week one), and (2) to ensure reduced energy intake during the restriction period (weeks two and three), subjects consumed meals designed and prepared by the Pennington Center Metabolic Kitchen. These meals had a fixed protein content of 1.3 grams/kg body weight. Subjects were instructed to eat no other food and to eat all of the food provided.

The protocol consisted of three weeks of physical training with pre and post energy restriction measurements of body composition, muscular strength and endurance, endurance performance, anaerobic capacity, and urinary nitrogen excretion:

(1) *Baseline Period (study days 1-10)*: Subjects reported to the Metabolic Kitchen for breakfast and dinner meals. Snacks and lunch were provided for off-campus consumption. Daily energy intake followed in accordance with each subject's individual energy needs to prevent weight loss or gain (i.e. eucaloric). In addition, subjects reported daily to the Exercise Testing Facility to perform treadmill exercise predetermined to elicit a 500 kcal energy expenditure.

(2) *Baseline measurements:* On day eight of the baseline period, subjects completed a muscle endurance test. On day nine, subjects completed tests for muscular strength and anaerobic performance tests. Tests on these days were performed either before or at least four hours after treadmill exercise. On day ten, lean body mass and percent body fat was determined by dual energy x-ray absorptiometry (DEXA). In place of that day's treadmill exercise bout, subjects participated in an endurance performance test. On days nine and ten all urine voids were collected and divided into two 24-hour periods for an assessment of 24-hour urinary nitrogen excretion.

(3) *Caloric Restriction Period (study days 11-24):* Subjects continued their daily treadmill exercise sessions, each bout sufficient to elicit a 500 kcal energy expenditure. Subjects continued to report to the Metabolic Kitchen for daily meals, however 16 (8 men and 8 women) out of the 24 had their total daily energy intake reduced by 750 kcal/day for 14 days (2 weeks). A majority of the energy deficiency came from withholding carbohydrate, while protein intake was held constant at 1.3 g/kg body weight. Thus, the percent of calories, which came from protein, was increased as total energy intake decreased. On day 12 of the energy restrictive phase, subjects repeated the muscle endurance test. On day 13, tests of muscle strength and anaerobic performance were repeated. On day 14, body composition by DEXA was re-assessed, and in place of the final treadmill exercise bout, subjects repeated the endurance performance test. On days 13 and 14 all urine voids were collected and divided into two 24-hour periods for a re-assessment of 24-hour urinary nitrogen excretion.

Eight subjects (5 men and 3 women) served as a control group. These subjects followed the protocol described above with one exception, on study days 11-24 they continued to consume a eucaloric diet. All subjects were blinded to the treatment (i.e., energy intake) during the experimental period.

Experimental Procedures

Maximal VO_2 Determination: Each subject was tested for max VO_2 on a treadmill using an incremental work protocol. Briefly, after establishing a sustainable pace (i.e., in minutes per mile), the treadmill incline was increased by 2% every minute until exhaustion while VO_2 and VCO_2 were continuously monitored with a SensorMedics Metabolic Cart (Vmax Series 29). Max VO_2 was defined as the highest 20 second average VO_2 achieved during the last 90 seconds of the test. Attainment of max VO_2 was accepted if two of the following three criteria were met: plateau in VO_2 with increasing workload, maximum heart rate within 10 beats of the age predicted maximum heart rate, and expiratory ratio greater than 1.10. Max VO_2 was used to evaluate pre-trial fitness level of the subjects.

To calculate average energy expenditure during running, after the subjects had a chance to recover from the max VO_2 test, they ran on the treadmill at a self-determined "comfortable" pace for 1 mile. Near the end of the run, expired air was analyzed for oxygen and carbon dioxide content using the SensorMedics metabolic cart. Energy expenditure per mile was calculated

from the oxygen consumption rate and respiratory exchange ratio. Energy expenditure per mile was calculated and used to determine the duration of treadmill exercise (at the set pace) during the baseline and energy restriction periods.

Energy Intake: Subjects reported to the Metabolic Kitchen for breakfast and dinner and these meals were eaten at the Pennington Center. Lunch (and snacks) was provided in a box to be consumed off campus. Subjects were instructed to consume all and only the food provided. Any amount not consumed was weighed, and the daily intake record was corrected. Energy intake was designed to meet each subject's individual energy needs as determined from the basal metabolic rate measurement (Harris-Benedict equation) and from the 3-day dietary record obtained during screening. During the baseline period, adjustments were made to account for the 500 kcal/day treadmill exercise energy expenditure. The diet was designed to include standard foods consumed in a regular American diet and meeting the nutritional recommendations of the US Recommended Dietary Allowances. One standard menu cycle (5 days) was designed for all study participants. Subjects were interviewed for individual food allergies or intolerance's, and food substitutions were made if necessary. Body weight was determined each morning prior to breakfast. During the energy restriction phase, subjects were not told their body weight.

Body Composition: Total body fat and lean body mass (LBM) were measured by dual energy x-ray absorptiometry (DEXA). The instrument used was a Hologic QDR 2000, operated with the Enhanced Array Whole Body Software Package, version 5.678A. DEXA determinations were made in the morning on days 10 of the baseline and 14 of the experimental periods.

Muscle Endurance Test: On day eight of the baseline and day 12 of the experimental period, a lower body muscular endurance test was completed. This test consisted of squatting exercise with a 100 lb weight for men and a 50 lb weight for women. The subjects performed 25 repetitions per minute, timed with a metronome, and continued till exhaustion. Exhaustion was defined as the point at which the subjects could no longer keep up with the metronome. Total number of repetitions for the test was recorded.

Muscle Strength Test: On day nine of the baseline and on day 13 of the experimental period, a one repetition maximum (1 RM) protocol was used (on *BodyMasters*TM resistance exercise equipment) to determine upper body (UB) and lower body (LB) strength. The Shoulder Press machine was used to determine UB strength and the Super Leg Press was used to determine LB strength. Standardized instructions explaining each test and a demonstration of proper technique was given prior to testing. After a standardized warm-up of 15 repetitions at a manageable resistance, a weight close to, but under the subject's expected maximum lifting capacity was selected. If one repetition was completed, 10 lbs increments were added to the exercise device until 1 RM was achieved.

Anaerobic Capacity Test: Following the muscle strength test on baseline day nine and experimental day 13, a Wingate anaerobic capacity test (Bar-Or, 1987) was performed. The test consisted of an all out 30 second maximal effort on a variable load CybexTM cycle ergometer at a

workload equal to 0.075 kg/kg body weight. Peak power was defined as the highest power output achieved in the first 5 seconds of a test, while mean power was defined as the average power output over the entire 30 seconds of a test.

Endurance Performance Test: In place of the treadmill exercise session on day ten of the baseline period and on day 14 of the experimental period, subjects performed a timed 5 mile running test designed to measure endurance performance. Subjects reported to the indoor Track at LSU's Student Recreation Center at assigned times. Instructions and information such as number of laps per mile, and proper lane usage followed a warm-up consisting of a 1 lap jog and standardized hamstring, quadriceps, groin and calf muscle stretches. Endurance performance was defined as each individual's 5 mile run time.

Urinary Nitrogen Excretion: On days nine and ten of the baseline period and on days 13 and 14 of the experiment, subjects collected all urine voids. The urine specimens were divided into two 24hr periods, the total volume measured, recorded and an aliquot saved for analysis of total nitrogen content and creatine by the Pennington Center's Clinical Laboratory.

Statistical Analysis: Data are presented as mean \pm SE. All data were analyzed by repeated measures analysis of variance with statistical significance set at $p < 0.05$.

Results

Energy Intake and Exercise Energy Expenditure: Energy intake in the control group was constant throughout the study averaging approximately 3200 kcal/day. Energy intake at baseline was similar between the control and energy restriction groups, but as planned, during experimental weeks one and two the restriction group consumed, on average, 720 fewer calories per day ($p < 0.05$). Exercise energy expenditure was slightly, but significantly (+ 23 kcal; $p < 0.05$) higher in the control vs. restriction group at baseline. This difference was maintained during experimental week one, but by experimental week two exercise energy expenditure was the same in the control and restriction groups, averaging 460 kcal/day. Thus, exercise energy expenditure was constant in the restriction group during baseline and the experimental periods; however, a small yet significant fall (- 22 kcal/day; $p < 0.05$) in exercise energy expenditure from experimental week one to week two was observed in the control group. Energy intake and exercise energy expenditure data can be found in Table 2.

Table 2. Energy Intake and Exercise Energy Expenditure (data are mean \pm SE)

	Baseline	Experimental Wk 1	Experimental Wk 2
<i>Control</i>			
<u>Energy Intake (kcal)</u>	3203 \pm 188	3248 \pm 198	3247 \pm 199
Exercise Energy			
Expenditure (kcal)	488 \pm 6*	482 \pm 8*	460 \pm 8

Energy Restriction

Energy Intake (kcal)	3124 \pm 153	2398 \pm 155*	2400 \pm 155*
Exercise Energy Expenditure (kcal)	465 \pm 7	463 \pm 6	459 \pm 9

* group x time interaction; $p < 0.05$

Body Weight: During the baseline period, body weight was stable in all subjects averaging 71.7 ± 2.4 kg. There was a significant group x time interaction for body weight change during the experimental period (* $p = 0.001$). As can be seen in Table 2, body weight loss for the energy restriction group averaged 1.2 kg, while weight loss for the control subjects was only 0.3 kg. Gender had no influence on these results.

Table 2: Change in body weight (kg) (data are mean \pm SE)

	Restriction Group	Control Group
Baseline Day 6	71.0 \pm 3.1	73.0 \pm 4.0
Baseline Day 10	71.0 \pm 3.2	73.0 \pm 3.9
Experimental Day 10	70.3 \pm 3.2	72.9 \pm 3.9
Experimental Day 14	69.7 \pm 3.2*	72.7 \pm 3.9

Body Composition and Nitrogen Excretion: A significant reduction in lean body mass but not body fat was observed. Further, there was a trend for the decrease in lean body mass to be greater in the restriction vs. the control group (group x time interaction; # $p = 0.09$). Body composition results are shown in the Table below. Gender had no influence on these results. Likewise, the increase in urinary nitrogen excretion was greater in the restriction group (i.e., +2.47 vs. +0.60 grams/24-hr, respectively), but this did not reach statistical significance ($p = 0.217$).

Table 3: Body Composition and Urinary Nitrogen Excretion Changes (data are mean \pm SE)

	Restriction Group		Control Group	
	Baseline	Experimental	Baseline	Experimental
Lean Body Mass (kg)	51.9 \pm 1.8	51.1 \pm 2.7#	55.7 \pm 4.1	55.6 \pm 3.9
Fat Mass (kg)	16.6 \pm 1.7	16.1 \pm 1.6	16.2 \pm 2.5	15.9 \pm 2.5
Urinary Nitrogen Excretion (grams/24hr)	9.85 \pm 0.92	12.32 \pm 0.68	10.59 \pm 1.02	11.19 \pm 1.01

Exercise Performance: Energy restriction did not impair measures of muscle endurance or 5 mile run time. In fact, when compared with baseline measures, muscle endurance and 5 mile run time was significantly improved following the experimental period (main effect of time; muscle endurance $p < 0.05$, 5 mile run time $p < 0.001$). Leg muscle strength was also improved following the experimental period without any negative effects of caloric restriction (main effect of time; $p < 0.05$). On the other hand, shoulder strength was unchanged in both groups during

the experimental period. Interestingly, there was a significant group x time interaction for anaerobic capacity. Both peak ($p < 0.05$) and average ($p < 0.05$) power output during the Wingate test were found to improve in the caloric restriction group while performance decrements were noted for control. Exercise performance values for all tests are presented in Table 4. Gender had no influence on the results.

Table 4: Exercise Performance (data are mean \pm SE)

	Restriction Group		Control Group	
	Baseline	Experimental	Baseline	Experimental
Muscle Endurance (repetitions)	73 \pm 16	88 \pm 19*	69 \pm 10	95 \pm 29*
5 Mile Run Time (seconds)	2566 \pm 72	2380 \pm 54†	2612 \pm 88	2475 \pm 77†
Leg Muscle Strength (lbs)	298 \pm 20	310 \pm 21*	324 \pm 29	330 \pm 28*
Shoulder Muscle Strength (lbs)	106 \pm 15	106 \pm 15	141 \pm 25	141 \pm 27
Peak Power (Watts)	598 \pm 35	634 \pm 38#	823 \pm 137	724 \pm 78
Average Power (Watts)	493 \pm 30	503 \pm 31#	564 \pm 51	546 \pm 31

* main effect of time; $p < 0.05$

† main effect of time; $p < 0.001$

time x treatment interaction; $p < 0.05$

Key Research and Accomplishments

- A laboratory model to study the effects of moderate energy restriction on physical performance of healthy young adults has been developed.
- Moderate (750 kcal/day), short-term (14 days) energy restriction in physically active young men and women results in weight loss. A majority of this weight loss comes from the lean body mass compartment.
- A dietary protein intake of 1.3 grams/kg does not appear adequate to prevent body nitrogen loss during short-term energy restriction in physically active young men and women.
- Moderate, short-term energy restriction in physically active young men and women does not impair indexes of physical performance.

Reportable Outcomes

- Two manuscripts which describe this research are in preparation.
- Julie Rickets received a Masters degree while being supported by this award.

C. Conclusions

Fourteen days of moderate energy restriction (750 kcal/day) in physically active young men and women results in body weight loss. A majority of this weight loss comes from the lean body mass compartment. Despite reductions in lean body mass, performance of specific exercise tasks which depend on muscular strength, power and endurance is not impaired.

Several subjects complained of muscle soreness and fatigue near the end of the first week of training (baseline period). This was likely the result of an increase in frequency (i.e., daily exercise training) of exercise training. Unfortunately, near the end of the first week is when we made our baseline measurements of physical performance. However we felt it was important to make the baseline measurements at this time because we wanted all subjects to be participating in a similar level (and frequency) of training when such measurements were made. Nonetheless, we may not have obtained a "true" baseline measurement of physical performance because of the existing level of fatigue. To better interpret the physical performance results, we will conduct another experiment in which measures of performance variables are made before and 7-10 days after increased frequency (daily) in exercise training.

D. References

1. Anderson, D.E. and Sharp, R.L. (1990). Effects of muscle glycogen depletion on protein catabolism during exercise. *Med Sci Sports Exer.* 22:(2, suppl), S59.
2. Askew, E.W., Claybaugh, J.R., Cucinell, S.A., Young, A.J., and Szeto, E.G. (1986). Nutrient intakes and work performance of soldiers during seven days of exercise at 7,200 ft. altitude consuming the meal, ready-to-eat ration. Technical Report No. T3-87. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
3. Baker-Fulco, C.J. (1995). Overview of dietary intakes during military exercises. Pp 121-149 in *Not Eating Enough*, B.M. Marriott, ed. A report of the Committee on Military Nutrition Research, Food and Nutrition Board, Institute of Medicine. Washington, D.C.: National Academy Press.
4. Bar-Or, O. (1987). The Wingate anaerobic test. A update on methodology, reliability, and validity. *Sports Med.* 4:381-394.

5. Butterfield, G.E. and Calloway, D.H. (1984). Physical activity improves protein utilization in young men. *Br J Nutr.* 51: 171-84.
6. Calloway, D.H. and Spector, H. (1954). Nitrogen balance as related to caloric and protein intake of active young men. *Am J Clin Nutr.* 2:405-11.
7. Cline, A.D. and Pusateri, A.E. (1996). Comparisons of iron status, physical activity, and nutritional intake of women entering Army officer and enlisted basic training. In *Proceedings of the 21st National Nutrient Databank Conference, Baton Rouge, June 1996.*
8. Cline, A.D., Warber, J.P., and Champagne, C.M. (1997). Assessment of meal consumption behaviors by physically active young men dining in a cafeteria setting. *FASEB J* Vol. 11, No. 3, 1070, p. A184.
9. Costill, D.L. (1988). Carbohydrate for exercise: Dietary demands for optimal performance. *Int J Sports Med.* 9: 1-18.
10. Edwards, J.S.A., Askew, E.W., King, N., Fulco, C.S., Hoyt, R.W., and Delany, J.P. (1991). An assessment of the nutritional intake and energy expenditure of unacclimatized U.S. Army soldiers living and working at high altitude. Technical Report No. T10-91, Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
11. Goranzon, H. And Forsum, E. (1985). Effect of reduced energy intake vs. Increased physical activity on the outcome of nitrogen balance experiments in man. *Am J Clin Nutr.* 41: 919-928.
12. Hirsch, E., Johnson, W., Dunne, P., Shaw, C., Hotson, N., Tharion, W., Lieberman, H., Hoyt, R., and Dacumos, D. (In Press). The effects of diet composition on food intake, food selection, and water balance in a hot environment. Technical Report. Natick, Mass.: Natick Research, Development and Engineering Center.
13. Interagency Board for Nutrition Monitoring and Related Research (1995). Third Report on Nutrition Monitoring in the United States, Vol. 2. Washington, D.C.: U.S. Govt. Printing Office. Life Sciences Research Office, Federation of American Societies for Experimental Biology.
14. King, N., Arsenault, J.E., Mutter, S.H., Champagne, C.M., Murphy, T.C., Westphal, K.A., and Askew, E.W. (1994). Nutritional intake of female soldiers during the U.S. Army basic combat training. Technical Report No. T94-17. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine .

15. Lemon, P.W.R. (1991). Effect of exercise on protein requirements. *J of Sports Sci.* 9: 53-70.
16. Lemon, P.W.R. and Mullin, J.P. (1980). Effect of initial muscle glycogen levels on protein catabolism during exercise. *J Appl Physiol.* 48: 624-9.
17. MacLean, D.A., Graham, T.E., and Spriet, L.L. (1989). Carbohydrate supply and amino acid and ammonia metabolism during prolonged exercise. *Med Sci Sports Exer.* 21: (2 suppl), S106.
18. Morgan, T.E., Hodges, L.A., Schilling, D., Hoyt, R.W., Iwanyk, E.J., McAninch, G., Wells, T.C., and Askew, E.W. (1988). A comparison of the Meal, Ready-to-Eat, Ration, Cold Weather, and Ration, Lightweight nutrient intakes during moderate altitude cold weather field training operations. Technical Report T5-89. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
19. Munro, H.N. (1951). Carbohydrate and fat as factors in protein utilization and metabolism. *Physiological Rev.* 31: 449-88.
20. NRC (National Research Council) (1989). Recommended Dietary Allowances, 10th ed. Report of the Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Washington, D.C.: National Academy Press.
21. Rose, M.S. and Carlson, D.E. (1986). Effects of A-ration meals on body weight during sustained field operations. Technical Report T2-87. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
22. Rose, R.W., Baker, C.J., Wisnaskas, W., Edwards, J.S.A., and Rose, M.S. (1989) Dietary assessment of U.S. Army basic trainees at Fort Jackson, South Carolina. Technical Report No. T6-89. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
23. Shippee, R., Friedl, K., Kramer, T., Mays, M., Popp, K., Askew, E., Fairbrother, B., Hoyt, R., Vogel, J., Marchitelli, L., Frykman, P., Martinez-Lopez, L., Bernton, E., Kramer, M., Tulley, R., Rood, J., Delany, J., Jezior, D., and Arsenault, J. (1994). Nutritional and immunological assessment of Ranger students with increased caloric intake. Technical Report No. T95-5. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.

24. Tharion, W.J., Cline, A.D., Hotson, N.G., Johnson, W., Niro, P., Baker-Fulco, C.J., McGraw, S., Skibinski, T.M., Shippee, R.L., Hoyt, R.W., DeLany, J.P., Tulley, R.E., Rood, J., Santee, W.R., Boquist, S.H.M., Bordec, M., Kramer, M., Slade, S.H., and Lieberman, H.R. (In Press). Nutritional challenges for field feeding in a desert environment: use of the UGR and a supplemental carbohydrate beverage. Technical Report, Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
25. Tharion, W.J., Hoyt, R.W., DeLany, J.P., Cline, A.D., and Lieberman, H.R. (1997). Effect of a carbohydrate drink on energy and fluid balance during a military field exercise in the desert. *FASEB J* Vol. 11, No. 3, 2558, p. A442.
26. Thomas, C.D., Friedl, K.E., Mays, M.Z., Mutter, S.H., Moore, R.J., Jezior, D.A., Baker-Fulco, C.J., Marchitelli, L.J., Tulley, R.T., and Askew, E.W. (1995) Nutrient intakes and nutritional status of soldiers consuming the meal, ready-to-eat (MRE XIII) during a 30-day field training exercise. Technical Report No. T95-6. Natick, Mass.: U.S. Army Research Institute of Environmental Medicine.
27. Walberg, J.L., Leidy, M.K., Sturgill, D.J., Hinkle, D.E., Ritchey, S.J., and Sebolt, D.R. (1988). Macronutrient content of a hypoenergy diet affects nitrogen retention and muscle function in weight lifters. *Int J Sports Med.* 9: 261-6.

V. Nutrient Database Integration Laboratory

A. Introduction

Timely receipt of dietary data is critical in assessing soldiers' nutritional needs and how those may interrelate with other aspects of military performance. We currently have diversified our tasks with planned field studies conducted by the Military Nutrition Division of USARIEM by providing analysis of dietary intakes collected during military field studies. We assist in the planning of dietary collection protocols and subsequent analysis of collected dietary intake data. We can advise the principal investigators on dietary collection and methods to assure a quick turnaround of nutrition information needed for statistical analyses. We are working with USARIEM nutrition staff, data programmers, and other key personnel in the development of a more effective database system for the collection and processing of dietary information. The objective is to generate finalized dietary data quickly, so that results can be disseminated in a timely fashion.

B. Body

Data from the dietary intake portion of the study "Effects of Tray Ration Consumption During a 63-Day Marine Field Exercise" was entered. The data was carried to PBRC from the PBRC personnel who participated in this study which took place in 1998 at Great Inagua Island

in the Bahamas during the periods from April 2-10 (Catherine Champagne), April 30-May 8 (Barbara Eberhardt) and May 21-29 (April Hebert). This data was entered and verified at PBRC and shipped to USARIEM. Unfortunately, Federal Express lost the raw data from the second phase of the study, but the entered data was reprinted and shipped to the USARIEM data collectors for verification by them. The amount of data in the total dataset was just under 20,000 lines.

The Nutrient Data Systems Section participated in a study under the direction of Dr. Matthew Kramer of Natick Labs with an Army unit at Fort Drum, New York in late summer of 1998. This protocol involved a field test to determine acceptability of MRE rations in a field exercise environment. The software used to process dietary information collected during field nutrition studies, MiDAS (acronym for Military Dietary Analysis System) was not used for this study.

Key Research Accomplishments

- Presentation of two abstracts at Experimental Biology '98 and one at Experimental Biology '99 resulting from previous participation in USARIEM studies.
- Participation in the Marines study "Effects of Tray Ration Consumption During a 63-Day Marine Field Exercise."
- Processing of data from the above study
- Participation in Natick Labs study conducted at Fort Drum, New York.
- Continued development of the MiDAS data collection and analysis system.
- Submission of manuscript "Incorporating new recipes into the Armed Forces Recipe File: Determination of acceptability by more cost effective means" to an appropriate journal.

Reportable Outcomes

The following abstracts were presented at the Experimental Biology '98 meetings:

DEVELOPMENT OF SPECIALIZED SOFTWARE TO FACILITATE COLLECTION OF DIETARY DATA AT REMOTE LOCATIONS. H.R. Allen, C.M. Champagne, and D.H. Ryan. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA. 70808.

Timely receipt of data via computerized nutrient analysis of dietary intakes of the soldiers is critical to assessment of soldiers' needs and interrelationships with other aspects of military life. In our Nutrient Database Integration Laboratory, we have developed a data-collection system to facilitate data entry and verify dietary records in off-site locations as one task identified under Military Nutrition Research. Dietary studies conducted on U.S. Army personnel

require that data collection software and computer equipment be mobile. A software application titled Military Dietary Assessment System (MiDAS) was developed to enable rapid collection of dietary data. MiDAS allows for data to be entered for each subject and sorted by date and meal. The data collection system consists of five notebook computers networked to provide a single shared database to store collected data. Data is verified in field by trained data collectors. Foods consumed are matched to foods in the USDA database upon return from the field allowing for rapid calculation of nutrient values for each study subject. MiDAS has been used in four field studies resulting in over 100,000 lines of consumption data from different types of data collection strategies. This system will be demonstrated and plans to integrate the Armed Forces Recipe File, special formulations and feedings into one database system that can address current and future Military Nutrition needs will be described. The plan is to develop a database that will be ever changing and modifiable to the unique set of circumstances presented by each study.

DIET AND CORONARY HEART DISEASE RISK FACTORS IN STUDENTS AT THE U.S. ARMY SERGEANTS MAJOR ACADEMY. C.M. Champagne, W.H. Karge, H.R. Allen, A.D. Cline, and C.J. Baker-Fulco. Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808 and U.S. Army Res.Inst.Env.Med., Natick, MA 01760.

Serum lipid levels and dietary habits were determined in 106 free-living students attending the U.S. Army Sergeants Major Academy (SMA). Dietary intake data was collected by 3-day food records upon entry at the SMA (Base), 10 weeks later (Mid), and after 26 weeks (End) as part of a health risk assessment and health education program for these career soldiers (average age 41 ± 4 years). Reported average intakes of energy, total fat, saturated fat, and dietary cholesterol for this group tended to decrease over the course of study. Average reported daily energy intake was 2351 kcal Base, 2275 kcal Mid, and 2080 kcal End. Total reported kcal from fat did not differ significantly at the three time periods (33.1% Base, 33.7% Mid, 31.4% End) with no appreciable changes in saturated fat kcal intake (11.1% Base, 11.3% Mid, and 10.6% End). Reported cholesterol intake tended to decrease with time (292 mg/day Base, 256 mg/day Mid, and 254 mg/day End). Serum lipid values associated with risk for CHD changed little over time and indicated that the average SMA soldier has total cholesterol (TC) levels placing them at moderate risk for CHD (TC = 200 mg/dl Base, 209 mg/dl Mid, 208 mg/dl End). The mean HDL levels ranged from 48.2 mg/dl at Base to 49.8 mg/dl at End. Triglyceride levels were 109 mg/dl Base, 102 mg/dl Mid, and 110 mg/dl End. Our data indicate that the average diet of these career soldiers does meet the American Heart Association (AHA) recommendations for dietary cholesterol intake (300 mg/day). We also noted a tendency to meet the AHA recommendations for dietary fat intake (30% kcal from fat with no more than 10% kcal from saturated fat). Further research is needed to determine factors that place them at moderate risk for CHD.

The following abstract, which used data from subjects from the Sargeants Major Academy study at Fort Bliss, TX, was presented at the Experimental Biology '99 meetings:

DAY-TO-DAY VARIATION IN FAT INTAKE: ESTABLISHING NORMATIVE DATA TO IDENTIFY POTENTIAL RISK FOR WEIGHT GAIN. C.M. Champagne, S.R. Smith, and W.A. Karge. Pennington Biomedical Research Center, Baton Rouge, LA 70808 and U.S. Army Research Institute for Environmental Medicine, Natick, MA 01760.

The capacity to adapt to a high fat diet under steady state conditions is remarkable. For example, it has been shown that athletes consuming a diet as high as 90% kcal from fat were able to maintain macronutrient balance. In free-living individuals, macronutrient content may vary greatly from day to day. During the shift from a low to a high fat diet, fat oxidation is slow to adapt. A positive fat balance is the result. As a consequence, large swings in dietary fat carry the hypothetical risk of promoting obesity. To test this hypothesis, knowledge of the intra-individual variation in dietary fat intake is required. We assessed reported dietary intake over 3 days in 100 middle age career soldiers (41 ± 4 years) who were enrolled in the Sergeants Major Academy. Mean fat intake was 86.8 ± 31.3 gm/day (range 20.3-175.3 gm/day) or $32.3 \pm 7.3\%$ of energy intake (range 10.2-49.9% of energy intake). The coefficient of variation (CV) within an individual ranged from 0.68% to 86.6% for a mean overall CV of $36 \pm 19.6\%$. The highest percentage of subjects (28%) had CVs within the range of 29.3% to 43.6%. Very high variations (58% to 87%) were noted in 13 individuals. A mean energy intake of 2380 ± 605 kcal/day was reported with 80% of the subjects having less than a 30% variation in reported mean energy intake. Reported protein and carbohydrate intakes appeared somewhat less variable. These results provide evidence that fat intake 1) varies widely within an individual, 2) that individuals differ in the variability of their fat intake, and 3) the swings in fat intake are of sufficient magnitude in some individuals to result in transient positive fat balances. Furthermore, we hypothesize that these transient positive fat balances due to variability in fat intake will promote fat mass gain.

The manuscript "Incorporating new recipes into the Armed Forces Recipe File: Determination of acceptability by more cost effective means" has been sent to Dr. Herb Meiselman at Natick Labs for consideration in the journal *Food Quality and Preference*. A copy of the manuscript is included in the Appendix.

C. Conclusions

The issues of software development, licensing, and other related matters discussed at the October visit to Natick continue to be addressed. We are coordinating efforts with USARIEM-Military Nutrition Division dietitians and data support personnel. Unfortunately, during this year our primary contact in the coordination of the server system at Natick was injured in an accident. A new contact at the USARIEM end has not yet been identified. We anticipate that efforts to create a more effective link with USARIEM will resume on a more full-scale basis when another computer person is named.

D. References

1. Champagne, C.M., W.H. Karge, H.R. Allen, A.D. Cline, and C.J. Baker-Fulco. Diet and coronary heart disease risk factors in students at the U.S. Army Sergeants Major Academy. FASEB J, 12(4):A224, 1998.
2. Allen, H.R., C.M. Champagne, and D.H. Ryan. Development of specialized software to facilitate collection of dietary data at remote locations. FASEB J, 12(4):A526, 1998.
3. Champagne, C.M., S.R. Smith, and W.A. Karge. Day-to-day variation in fat intake: establishing normative data to identify potential risk for weight gain. FASEB J, 13(4):A264, 1999.

VI. Enhancing Military Diets

A. Introduction

The current task has evolved from development of heart healthy recipes for the Armed Forces Recipe File to assessment of food selection practices of military basic training students. Upon request from the Army Chief Dietitian, OTSG, we have modified the task to re-direct our involvement to include analysis of current eating practices of young military members and intervention to improve nutritional status as needed. This change has come about at the time that the Armed Forces Recipe Service has indicated that they no longer need development of modified recipes, due to a change in their food procurement system. We will apply marketing and behavioral theories, service member and staff feedback, and current foodservice industry innovations to a nutrition program that will result in positive changes in consumption to meet the nutritional recommendations of increased food sources of calcium, antioxidants, and folate. To accommodate unique requirements of each of the military services, we will design innovative programs that address concerns of each service while accomplishing similar nutritional goals for all.

B. Body

Food preference surveys were distributed in dining facilities at 9 military installations to 2818 individuals; 2538 were returned (90%). Gender distribution was 81.4% male, 18.6% female; 60% were in the 18-21 y.o. range. All areas of the country were represented in geographic distribution of respondents (Table 1).

Table 1. Description of military respondents to food preference survey by gender (n=2538)

Category	Male	Female
Gender	2063	475
Age (years)		
18-21	1196	332

22-25	541	96
26-29	165	26
30 and over	161	21
Ethnic Origin		
Caucasian	1281	257
African American	314	127
Asian	73	12
Hispanic	228	47
Am. Indian	34	3
Other	121	26
Geographic Breakdown		
Middle Atlantic	318	74
East North Central	278	64
East South Central	130	28
West South Central	291	71
Pacific	257	58
New England	84	27
West North Central	114	30
Atlantic	377	76
Mountain	102	23
Other	68	20

Major findings include the following: more frequent selection of "low-calorie/diet food" by women (33% vs 19% daily); more frequent selection of "power/energy food" by men (33% vs 23%); no differences in selection of "fast food/convenience food" (men - 12%, women - 10% daily), or "heart healthy/low cholesterol food" (men - 34%, women - 35%) (Table 2).

Table 2. Gender differences in frequency of selection of specific food categories (n=2471)

Food Category	Male %	Female %
Fast/convenience		
Once a week	40.3	40.3
3-4 times/week	38.3	37.1
once a day	8.9	5.6
twice a day	4.2	4.5
none of the above	8.0	11.8
Diet/low calorie		
Once a week	24.6	22.8
3-4 times/week	22.2	26.0
once a day	10.9	17.8
twice a day	7.9	13.0
none of the above	34.2	20.0
Heart healthy		
Once a week	22.2	23.7
3-4 times/week	29.4	28.0
once a day	15.4	15.9
twice a day	14.7	17.4
none of the above	18.3	15.0
Power/energy		
Once a week	20.2	25.3
3-4 times/week	26.2	19.0

once a day	17.6	13.3
twice a day	12.6	7.8
none of the above	23.4	34.2

Gender differences in food preference include: vegetarian (women - 5.8 rating, men - 4.5 rating); women preferred chicken to beef and pork, men gave higher ratings for spicy foods, women gave higher ratings to non-egg breakfast items, low acceptability of baked and broiled fish by both genders; top ethnic food choices were Italian (men - 7.1 rating, women - 7.1 rating), Mexican (men - 6.8 rating, women - 6.8 rating), and Chinese (men - 6.4 rating, women - 6.8 rating). Differences in responses by individual base can be found in the Appendix.

A food consumption and acceptability study was conducted at Ft. Lee, VA, Oct. 27-30, 1998. Data were collected on food selection and acceptability by gender at two dining facilities. Participants in the survey included military students who are required to consume all meals at the facility and instructors assigned to the installation who accompany student classes during the duty day. Number of participants surveyed were: Control (Thu) Breakfast - 57, Lunch - 198, Dinner - 69; Intervention (Fri) Breakfast - 411, Lunch - 446. PBRC recipes were incorporated into the menu at both locations; the control facility did not differentiate between the PBRC and Armed Forces recipes on the serving line. The intervention facility incorporated the "Power Performance" nutrition education program into materials placed throughout the dining area. PBRC food items were identified with a "Power Performance" logo and nutrient information. Data analysis ascertained gender differences in food selection, acceptability scores of foods consumed, and importance of nutrition information in food choices.

Results are as follows:

- Selection of short order items – control facility, 68.2% of diners; intervention facility, 65.7% of diners
- Survey response to selection of low-fat items – control facility, 34.3% select regularly; intervention facility, 37.1%
- Selection of "Power Performance" entrée – control facility, 24.2%; intervention facility, 19.5% - fewer took entrée when identified as fat-modified
- Majority of respondents did not notice nutrition information posted in dining facility

Table 3. Hedonic Ratings of Modified Recipes Control vs Intervention Facility by Gender

Food Item	Gender Selection - Control Facility		Gender Selection - Intervention Facility		Rating (M)		Rating (F)	
	M (%)	F (%)	M (%)	F (%)	Control	Intervention	Control	Intervention
Quesadilla	25(75.8)	8(24.2)	6(100)	0(0)	6.8	6.0	5.9	-
Chicken Broccoli Pasta	19(63.3)	11(36.7)	41(64.1)	23(35.9)	8.2	8.3	7.7	8.0
Roasted Pepper Potatoes	8(66.7)	4(33.3)	11(55.0)	9(45.0)	7.1	7.3	5.8	6.9

Greek Pasta Salad	1(50.0)	1(50.0)	22(66.7)	11(33.3)	7.0	5.6	8.0	4.7
Chicken Okra Gumbo	2(33.3)	4(66.7)	21(91.3)	2(8.7)	6.0	7.8	6.0	7.5

There were no significant differences between gender or days in hedonic ratings of modified recipes.

Key Research Accomplishments

- Data are now available on food selection by gender on a limited number of food categories. These will enable research teams to complete further analysis in the future to determine nutritional composition of meals consumed by service members in a cafeteria setting.

Reportable Outcomes

A poster was presented by Alana Cline at the Annual Meeting of the American Dietetic Association, Kansas City, MO, October 1998. Title of the abstract was: "Gender differences in food preferences of young men and women in the Armed Forces. AD Cline, HR Allen, K Patrick, AE Hunt."

C. Conclusions

Food selection practices of young military members are similar to civilian counterparts of the same age. As selections change, nutrition intervention will need to focus on educating service members, dining facility managers, and food purchasing personnel to assure that food selections meet nutritional requirements for performance.

D. References

1. Kluter RA, Leshner LL, Vanca GT. The 1992-1993 Army Food Preference Survey. US Army Research, Development and Engineering Center, Natick, MA: Technical Report TR-95/011, 1994.

VII. Stress, Nutrition and Immune Function Laboratory

A. Introduction

Participation in physically rigorous military training results in both physical and mental stress [1]. Some of the stressors associated with training include caloric deficiencies, sleep deprivation and exercise. Concomitant with these stresses is an alteration of a number of parameters of lymphocyte-related immunity [2-4]. Though the significance of these *in vitro* alterations in immune responses is unclear [4], an increase in documented infection rates coincided with indications of compromised immune function in US Army Rangers course

participants [5] . Thus the characterization of the mechanism of stress-induced alterations in immune responses could lead to the development of preventative strategies for reducing this immunomodulatory effect and improving the health of the soldiers. While the precise mechanism of this altered immune responses remains unknown, it is likely that inadequate energy intake contributed to this phenomena [3] .

The nutritional status of an individual can be a very important component of the stress response, either being directly responsible for the stress [6, 7] or affecting the response to other stressors [8, 9] . While moderate dietary energy restriction prolongs life-span and enhances immune responsiveness [10] , protein-energy malnutrition is associated with decreased lymphocyte proliferation reduced cytokine release, and lower antibody response to vaccines [7] and enhancement of tumor metastasis [11] . On the other hand, excessive intake of nutrients also impairs immunity [12] . Polyunsaturated fatty acids (PUFA) are structurally important for cell membranes and play a significant role as precursors (esp. arachidonic acid) of eicosanoids (prostaglandins, thromboxanes, leukotrienes). However, these eicosanoids may produce exaggerated effects in acute stress responses causing immunosuppression [8] . The rate of eicosanoid synthesis is determined by PUFA turnover and this is enhanced stressful conditions such as trauma and sepsis [13] . The nature of eicosanoids produced is determined by the availability of different PUFA in the cellular phospholipid pool. N-6 and n-3 PUFA give rise to different eicosanoids [13] with the eicosanoids formed from n-3 PUFA seeming to be more beneficial to the immune system compared to those derived from n-6 PUFA [14] . This has led to the suggestion that the amount and type of dietary fatty acids can influence *in vitro* measures of immune function [15] and that dietary composition may need to be altered during stressful conditions [16] .

We have developed a sleep-deprivation (SD) model of stress-induced immune modulation. Rats subjected to sleep-deprivation exhibit profound evidence of physiologic stress and an alteration in their immune system. Thus in several ways this stress model mimics some of the changes observed in the Army Rangers' training course [3] . During this past year our efforts have focused on determining the effect of dietary fatty acid on sleep-deprivation induced immune deviation in the rats. Immune function was determined both using *in vitro* and *in vivo* measures. Preliminary studies characterized the effect of different dietary fatty acids on immune function. Subsequent studies determined the effect of the diets on sleep-deprivation induced immune modulation. Our results were both consistent with prior reports regarding the effect of dietary fat on baseline immune function and also novel in regards to the immunoprotective nature of some of the diets.

B. Body

Materials and Methods

Animals. Male Sprague-Dawley rats (600 g) were used in all experiments. Rats were caged individually with *ad libitum* access to food and water.

Sleep Deprivation. Rats were sleep deprived by placing them into pedestal-style cages in which a small platform is surrounded by water. Rats are allowed free access to food and water while on the platform but they cannot lie down. Rats were subjected to sleep deprivation for 24 - 72 hours.

Diet. Rats were fed either a low fat diet (LF), a safflower oil diet (SO) high in n-6 polyunsaturated fatty acids (PUFA), an olive oil diet (OO) high in monounsaturated fatty acids, a coconut oil diet (CO) high in saturated fatty acids, and a menhaden oil (MO) diet high in n-3 PUFA. All diets contain 4% corn oil in addition to the other oils which represented 30% of the calories in each of the diets.

Experimental Design and Analysis. Rats were placed onto the test diet two weeks prior to stressing and remained on the diet throughout the stress period. Four rats were included in each diet group and splenocyte cultures were set up in quadruplicates. Results represent the average counts per minute (CPM) for the 4 rats. Results were analyzed using an one-way analysis of variance with post hoc analysis using Student Neuman Keuls multiple comparison test.

Lymphocyte proliferation. Splenic lymphocytes (2×10^5) were incubated for three days with mitogens (pokeweed (PWM), phytohemagglutinin (PHA) and concanavalin A (ConA) with or without recombinant IL-2 for three days at 37° C in a CO₂ incubator. Plates were pulsed for 4 hours with 0.5 μ Ci of ³H-thymidine and the DNA harvested onto filter pads for liquid scintillation counting. Stimulation indices (SI) were calculated as CPM of stimulated cultures/ CPM of medium controls. All determinations were performed in triplicate.

Results

As seen in Figure 1 (Appendix), and reported by other groups [17, 18], there was a significant difference ($p < 0.001$) in ³H-thymidine incorporation among the different diets. Those rats fed the SO and CO diets exhibited the highest CPM, whereas those on the LF, MO and OO diets were the lowest for those cells cultured in autologous sera (Figure 1). Incubating the cells in media containing FCS affected these results in two ways (Figure 2) (Appendix). First, there was an overall increase in the lymphoproliferative response in the ConA-stimulated cultures, with a less dramatic effect on the PWM cultures and an inhibition of the PHA response. Second, while SO and CO remained the highest responders in the ConA cultures, they were not different from LF in the PHA- and PWM-stimulated responses. The OO and MO cultures still yielded the lowest results in terms of ³H-thymidine incorporation. While earliest work indicated that the effects of dietary lipid manipulation were totally reversed when lymphocytes were cultured in FCS [19], we noted a less dramatic change.

To determine the effect of diet on the SD-induced suppression of the lymphoproliferative response to mitogens, rats were pre-fed the diets for two weeks and either sleep deprived for 48

hours (SD) or kept in their cages (CC). As seen in the preliminary studies, there was a significant ($p < 0.05$) dietary effect on the lymphoproliferative response of the CC rats fed the MO diet (Figure 3). Sleep deprivation of the rats on the LF diet also resulted in a characteristic suppression of their immune response to PHA ($p < 0.05$). Similar effects were seen in response to ConA and PWM (data not shown). Those rats on the OO and CO diets also exhibited reduced, though not significant, lymphoproliferative responses following sleep-deprivation. By contrast those rats on the MO and SO diets had significantly ($p < 0.01$) augmented proliferative responses compared to the CC rats on the same diet.

Key Research Accomplishments

- Demonstrated the immunosuppressive and anti-inflammatory effects of PUFA in animal experiments.
- Demonstrated that sleep deprivation stress produces immunosuppression and anti-inflammatory response in rats fed low-fat olive oil or coconut oil diets, but not those fed menhaden oil or safflower oil.

C. Conclusions

As shown here, and reported by others [17, 18], diets rich in polyunsaturated fatty acids (PUFA), especially n-3 PUFA, are anti-inflammatory and immunosuppressive. The splenocytes from the FO and SO fed control rats exhibited decreased proliferative responses to the mitogens. This effect was most apparent when the splenocytes were cultured in media containing autologous sera, though similar results were obtained with the cells cultured in FCS. A prior study had indicated an even greater effect of FCS on these responses [19]. Nevertheless, it is clear that the addition of FCS to the cultures does alter the dietary effects and thus autologous sera will be used in all future studies.

The mechanism of dietary fat effects on immune function remain uncertain. While alterations of eicosanoid pathways are influenced by n-3 PUFA, it is also clear that these fatty acids can also elicit their effects by eicosanoid-independent mechanisms [20]. Alterations in CD4:CD8 ratios have been associated with feeding diets high in n-3 PUFA [21]. While we did not examine lymphocyte subset distributions in the current studies, future studies will address this question. Likewise possible effects of the diet on macrophage function were not assessed, though others have reported significant alterations [22].

A somewhat surprising attribute of dietary supplementation with PUFA is the prevention of immunosuppression after surgical [23, 24] and burn trauma [25]. Given the immunosuppressive nature of these diets, such a protective effect seems paradoxical. Nevertheless a similar effect of dietary fat on exercise-induced immune modulation has also been reported [26]. Though these findings remain controversial [27, 28], no systematic analysis of dietary fatty acids on stress-induced changes in immune function has been reported. Here we

report that rats fed either LF, OO, or CO diets for two weeks and then subjected to sleep deprivation exhibited signs of SD-induced immunomodulation as evidenced by decreased *in vitro* lymphoproliferative responses to the mitogens tested. Following sleep deprivation, only splenocytes from the LF, CO and OO fed rats exhibited decreased lymphoproliferation. Those rats fed the MO diet exhibited an augmentation of their lymphoproliferative response, as compared to the unstressed cage controls (CC). While this could be attributed to alterations in the eicosanoid pathway leading to reduced levels of E series prostaglandins, it does not explain the similar effect seen in the rats fed the SO diet. An eicosanoid-independent pathway appears likely and remains to be identified.

Our reason for focusing on a high fat diet was two-fold. First, there is a considerable body of data indicating that dietary fats can influence immune responses [29-33], particularly during periods of stress [16, 34]. Second, since caloric deficiencies associated with military training is thought to be a contributor to the alterations in immune function [2, 3], the incorporation of high caloric foods to combat these deficiencies may be contemplated [35]. As such the results of these studies should be of particular interest to the Army since they may both identify the mechanism of stress-induced immune modulation and provide a rational basis for potential therapeutic (dietary) intervention.

D. References

1. Bernton E, Hoover D, Galloway R, Popp K. Adaptation to chronic stress in military trainees. Adrenal androgens, testosterone, glucocorticoids, IGF-1, and immune function. *Ann N Y Acad Sci* 1995;774:217-31
2. Boyum A, Wiik P, Gustavsson E, Veiby OP, Reseland J, Haugen AH, Opstad PK. The effect of strenuous exercise, calorie deficiency and sleep deprivation on white blood cells, plasma immunoglobulins and cytokines. *Scand J Immunol* 1996;43:228-35
3. Kramer TR, Moore RJ, Shippee RL, Friedl KE, Martinez-Lopez L, Chan MM, Askew EW. Effects of food restriction in military training on T-lymphocyte responses. *Int J Sports Med* 1997;18 Suppl 1:S84-90
4. Lee DJ, Meehan RT, Robinson C, Mabry TR, Smith ML. Immune responsiveness and risk of illness in U.S. Air Force Academy cadets during basic cadet training. *Aviat Space Environ Med* 1992;63:517-23
5. Martinex-Lopez L, Friedl K, Moore R, Kramer T. A longitudinal study of infections and injuries of Ranger students. *Mil. Med.* 1993;158:433-437
6. Nakamura K, Aoike A, Hosokawa T, Rokutan K, Koyama K, Nishi Y, Yoshida A, Kawai K. Effect of food-restriction stress on immune response in mice. *J Neuroimmunol* 1990;30:23-9

7. Lesourd BM. Nutrition and immunity in the elderly: modification of immune responses with nutritional treatments. *American Journal of Clinical Nutrition* 1997;66:478S-484S
8. Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. *Int J Vitam Nutr Res* 1995;65:3-20
9. Franci O, Amici A, Margarit R, Merendino N, Piccolella E. Influence of thermal and dietary stress on immune response of rabbits. *J Anim Sci* 1996;74:1523-9
10. Meydani SN, Lipman R, Blumberg JB, Taylor A. Dietary energy restriction decreases ex vivo spleen prostaglandin E2 synthesis in Emory mice. *Journal of Nutrition* 1990;120:112-5
11. Hodgson DM, Chiappelli F, Kung M, Tio DL, Morrow NS, Taylor AN. Effect of acute dietary restriction on the colonization of MADB106 tumor cells in the rat. *Neuroimmunomodulation* 1996;3:371-80
12. Chandra RK, Kumari S. Nutrition and immunity: an overview. *Journal of Nutrition* 1994;124:1433S-1435S
13. Suchner U, Senfleben U. Immune modulation by polyunsaturated fatty acids during nutritional therapy: interactions with synthesis and effects of eicosanoids. *Infusionsther Transfusionsmed* 1994;21:167-82
14. Gogos CA, Ginopoulos P, Zoumbos NC, Apostolidou E, Kalfarentzos F. The effect of dietary omega-3 polyunsaturated fatty acids on T-lymphocyte subsets of patients with solid tumors. *Cancer Detection & Prevention* 1995;19:415-7
15. Rasmussen LB, Kiens B, Pedersen BK, Richter EA. Effect of diet and plasma fatty acid composition on immune status in elderly men. *American Journal of Clinical Nutrition* 1994;59:572-7
16. Hannigan BM. Diet and immune function. *British Journal of Biomedical Science* 1994;51:252-9
17. Jeffery NM, Yaqoob P, Newsholme EA, Calder PC. The effects of olive oil upon rat serum lipid levels and lymphocyte functions appear to be due to oleic acid. *Annals of Nutrition & Metabolism* 1996;40:71-80
18. Yaqoob P, Newsholme EA, Calder PC. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology* 1994;82:603-10

19. Yaqoob P, Newsholme EA, Calder PC. Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. *Biochimica et Biophysica Acta* 1995;1255:333-40
20. Calder PC. N-3 polyunsaturated fatty acids and immune cell function. *Adv Enzyme Regul* 1997;37:197-237
21. Gogos CA, Ginopoulos P, Zoumbos NC, Apostolidou E, Kalfarentzos F. The effect of dietary omega-3 polyunsaturated fatty acids on T- lymphocyte subsets of patients with solid tumors. *Cancer Detect Prev* 1995;19:415-7
22. Hughes DA, Southon S, Pinder AC. (n-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes in vitro. *J Nutr* 1996;126:603-10
23. Blackburn GL. Nutrition and inflammatory events: highly unsaturated fatty acids (omega-3 vs omega-6) in surgical injury. *Proceedings of the Society for Experimental Biology & Medicine* 1992;200:183-8
24. Ayala A, Chaudry IH. Dietary n-3 polyunsaturated fatty acid modulation of immune cell function before or after trauma. *Nutrition* 1995;11:1-11
25. Tashiro T, Yamamori H, Takagi K, Hayashi N, Furukawa K, Nakajima N. n-3 versus n-6 polyunsaturated fatty acids in critical illness. *Nutrition* 1998;14:551-3
26. Benquet C, Krzystyniak K, Savard R, Guertin F, Oth D, Fournier M. Modulation of exercise-induced immunosuppression by dietary polyunsaturated fatty acids in mice. *Journal of Toxicology & Environmental Health* 1994;43:225-37
27. Peck MD. Omega-3 polyunsaturated fatty acids: benefit or harm during sepsis? *New Horiz* 1994;2:230-6
28. Mendez C, Jurkovich GJ, Garcia I, Davis D, Parker A, Maier RV. Effects of an immune-enhancing diet in critically injured patients. *J Trauma* 1997;42:933-40; discussion 940-1
29. Calder PC. Fatty acids, dietary lipids and lymphocyte functions. *Biochemical Society Transactions* 1995;23:302-9
30. Crevel RW, Saul JA. Linoleic acid and the immune response. *European Journal of Clinical Nutrition* 1992;46:847-55
31. Hummell DS. Dietary lipids and immune function. *Progress in Food & Nutrition Science* 1993;17:287-329

32. Katan MB, Zock PL, Mensink RP. Effects of fats and fatty acids on blood lipids in humans: an overview. *American Journal of Clinical Nutrition* 1994;60:1017S-1022S
33. Kinsella JE, Lokesh B. Dietary lipids, eicosanoids, and the immune system. *Critical Care Medicine* 1990;18:S94-113
34. Gerster H. The use of n-3 PUFAs (fish oil) in enteral nutrition. *International Journal for Vitamin & Nutrition Research* 1995;65:3-20
35. Pendergast DR, Horvath PJ, Leddy JJ, Venkatraman JT. The role of dietary fat on performance, metabolism, and health. *American Journal of Sports Medicine* 1996;24:S53-8

VIII. Metabolic Unit Project

A. Introduction

The purpose of this task is twofold. First, task provides clinical services to support Task 4 and Task 7. The second task is to apply the results of the basic science research performed in Task 4, 7, and 3 to human nutritional biology.

B. Accomplishments

The accomplishments of this task pertain to the support role for Tasks 4 and 7. Specifically, Task 4 has completed several research protocols that have utilized the resources of this task. Please see report of Task 4 for details. In addition, this task has completed a pilot project in preparation for clinical testing of results obtained under Task 7. Specifically, we have refined a new method to simultaneously measure gene expression (mRNA) for several hundred cytokine-related genes in a single sample. We expect this technique, cDNA microarray hybridization, will be applied to test hypothesis related to stress / nutrition / immune system interactions in upcoming human studies of immune function. We are currently in the process of scaling up this technique to provide a greater number of mRNAs that can be measured in a single RNA sample.

Key Research Accomplishments

- The development of methods to measure multiple mRNAs in a sample by cDNA microarray technology.

C. Conclusions

This task provided a role in support of the other clinical tasks during the calendar year. We expect that the techniques available within this task will result in the planning and initiation of a clinical trial within the next fiscal year. This clinical research trial will apply the results of the body of knowledge gained from the efforts of the other tasks.

D. References

None applicable.

APPENDICES

APPENDIX I

OVERVIEW



Pennington Biomedical Research Center
LOUISIANA STATE UNIVERSITY

July 16, 1998

Dr. Harris R. Lieberman
Grant Officer's Representative
Chief, Military Nutrition Division
U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

Subject: Cooperative Agreement DAMD 17-97-2-7013
Request to Purchase Equipment

Dear Dr. Lieberman:

In order to provide you effective and timely field support for collecting and analyzing the Army nutrient data, approval is hereby requested to purchase the attached itemized list of equipment (attachment 1).

Justification for the purchase of equipment was provided by Dr. H. Raymond Allen who is a member of Dr. Alana Cline's team that will be coordinating data entry protocols for the field study, as well as compiling the final data report for the principal investigator at USARIEM. Attachment 2

Additionally, it is requested that retroactive approval be provided for a 5 quart mixer and a 12 quart mixer. The small mixer is needed for preparation of smaller recipes, while the larger mixer is required for recipes serving 100 or more that are being developed for the Army. The small mixer costs \$1,475, and the large mixer costs \$2,713.

Your approval is urgently requested so that we can have the proper equipment for our next field study scheduled to take place in the near future.

Sincerely,

Patrick J. Marquette
Director of Sponsored Projects

PJM/ad

c: Mr. B. C. Baker, III
Dr. Ryan
Dr. York
Mr. Silvia
Ms. Fisher

Approved by Dr. Donna H. Ryan,
Principal Investigator

Equipment Request

Item	Quant	Cost	Total
Dell Latitude LM Notebook Computer (233 MHz)	2	\$2,399.50	\$4,799.00
Dell 266 MHz Latitude CP Notebook Computer	2	\$3,278.96	\$6,557.92
3Com 3C689 Tokenlink III 16/4 PC Card	4	\$311.25	\$1,245.00
3Com 3C589D-TP Etherlink III LAN PC Card	4	\$123.00	\$492.00
3Com OfficeConnect Hub 8 Port	1	\$89.95	\$89.95
Category 5 Patch Cables 10 foot length	6	\$10.95	\$65.70
Keyboards	4	\$34.95	\$139.80
Microsoft Mouse	4	\$59.95	\$239.80
SPSS 8.0	2	\$895.00	\$1,790.00
Microsoft Office 97	3	\$200.00	\$600.00
Microsoft SQL Server	1	\$2,000.00	\$2,000.00
Canon BJC-250 Bubblejet Printer	1	\$150.00	\$150.00
HP Scanjet 5S Scanner	1	\$200.00	\$200.00
Remark Office OMR 4.0	1	\$449.00	\$449.00
			<u>\$18,818.17</u>

~~800 phone line @ \$0.13/minute~~

\$50/month

WAK

Approved DAK

PENNINGTON BIOMEDICAL RESEARCH CENTER
LOUISIANA STATE UNIVERSITY

Interoffice Correspondence

From: H. Raymond Allen, Ph.D.

Date: 07/15/98

To: Patrick Marquette

Re: Computer Equipment for Dr. Alana Cline

Per your request, I have generated a list of equipment which will allow Dr. Cline to collect and analyze data in the field. The equipment consists of 3 notebook computers, networking equipment, a scanner, printer, and software.

Additionally, I am requesting one new notebook computer for the MENu database project. The field collection software used with our application MiDAS needs to be updated to use Microsoft SQL database. In order to do this we must have a notebook computer which can run Microsoft NT v4.0. The notebook computers originally purchased on the Army grant are not capable of running this operating system.

The Microsoft SQL Server software is requested to complete installation of MiDAS on the server located at USARIEM. Completion of this installation needs to be completed within the next two months.

Finally, there is a need to have an 800 number connected to the MENu database system to facilitate the transfer of Nutrient data both between PBRC and USARIEM and also from field locations to PBRC. The cost of an 800 line is based on usage with the current cost being \$0.13/minute. I am estimating a maximum cost of \$50/month to establish this line.

Attached is a detailed list of the equipment requested.

The 800 number must be charged
to overhead.

DSH/Gar 7/14/98



DEPARTMENT OF THE ARMY

US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

December 8, 1998

REPLY TO
ATTENTION OF:

Research Data Management

SUBJECT: Review of Annual Report Dated July 1998,
Award Number DAMD17-97-2-7013

Donna H. Ryan, M.D.
Pennington Biomedical Research Center
Louisiana State University
6400 Perkins Road
Baton Rouge, Louisiana 70808-4124

Dear Doctor Ryan:

Subject report has been reviewed and is acceptable as written. A copy of your report has been forwarded to the Defense Technical Information Center's Technical Reports database.

Point of contact for this action is Ms. Judy Pawlus at 301-619-7322 or email judy_pawlus@ftdetrck-ccmail.army.mil.

Sincerely,

Judy Pawlus
Office of the Deputy Chief of
Staff for Information Management



Pennington Biomedical Research Center

LOUISIANA STATE UNIVERSITY

6400 Perkins Road; Baton Rouge, Louisiana 70808-4124

Phone: (504) 763-2500

FAX: (504) 763-2525

TELEFAX COVER SHEET/LETTER

Date: 11/16/98 No. of Pages (Including Cover) 5
TO: Major Tobias FAX No. (504) 233-4869
FROM: Juice Warren Account No. _____
RE: _____

MESSAGE: Major Tobias - here is a copy of
what I FAXed to Major Public today

SIGNED: _____

c: _____

Original Document Will Follow By Mail ☐

Original Document Will Not Follow By Mail ☒

CONFIDENTIALITY NOTICE: THIS FACSIMILE TRANSMISSION AND THE DOCUMENTS ACCOMPANYING IT MAY CONTAIN WORK PRODUCT AND/OR PRIVILEGED AND CONFIDENTIAL INFORMATION INTENDED ONLY FOR THE USE OF THE INDIVIDUAL OR ENTITY NAMED ABOVE. IF THE READER OF THIS MESSAGE IS NOT THE INTENDED RECIPIENT, YOU ARE HEREBY NOTIFIED THAT ANY DISSEMINATION, DISTRIBUTION OR COPY OF THIS TELECOPY IS STRICTLY PROHIBITED. IF YOU HAVE RECEIVED THIS TELECOPY IN ERROR, PLEASE IMMEDIATELY NOTIFY US BY TELEPHONE AND RETURN THE ORIGINAL MESSAGE TO US AT THE ABOVE VIA THE U.S. POSTAL SERVICE. THANK YOU.



Pennington Biomedical Research Center

LOUISIANA STATE UNIVERSITY

6400 Perkins Road, Baton Rouge, Louisiana 70808-4124

Phone: (225) 763-2500 FAX: (225) 763-2525

TELEFAX COVER SHEET/LETTER

Date: 11/16/98 No. of Pages (Including Cover) 4

To: Major David L. Ruble FAX No. (301) 619-4165
Company: Animal Care and Use Review

From: Donna H. Ryan, M.D. Account No. _____

Re: DOD Animal Use Reporting Requirements

MESSAGE : Attached are the materials which you requested:

1. Animal Use Report for two grants
2. USDA Inspection Report

Please call me at 225-763-2514 if you have any questions.

SIGNED: _____

c: _____ Donna Ryan

Original Document Will Follow By Mail ☐

Original Document Will Not Follow By Mail ☐

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DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

November 2, 1998

Animal Care & Use Review Division

Subject: DOD Animal Use Reporting Requirements

Donna M. Ryan
Louisiana State University
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, LA 70808

Dear Investigator:

Reference your grant/contract with the United States Army Medical Research & Materiel Command (USAMRMC). Congress has mandated that the Department of Defense (DOD) collect data on animal use in DOD-sponsored research. Other regulations require that a copy of the **most recent U.S. Department of Agriculture (USDA) Inspection Report (APHIS Form 7008 or equivalent, with attachments)** be reviewed on an annual basis for all sponsored facilities.

Please provide this office with:

- a. A completed animal use report (a reproducible copy of the form is enclosed). This report should contain **only data relevant to your grant or contract with the USAMRMC**. A separate report must be filed for each contract or grant under which you use animals, regardless of where the work was performed.
- b. A copy of the **most recent USDA inspection report(s)** for the institution in which you conduct animal work and/or any subcontracting facilities.

The Chair of your Institutional Animal Care and Use Committee and/or your Attending Veterinarian should be able to provide assistance in meeting these requirements. Prompt compliance with this request will help to prevent funding delays; please submit them **immediately**. These documents are required annually and are **due on December 1** for the period covering the prior government fiscal year, October 1 through September 30. If you have already sent both of these documents, please disregard this letter. However, if you have only sent one of these documents, please provide the other document, or both documents as appropriate, to U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RCQ-AR (Major Ruble), 504 Scott Street, Fort Detrick, MD 21702-5012. Documents may be FAXED to this office at 301-619-4165.

If you have any questions, please contact this office at (301) 619-2144, or Email the undersigned at MAJ.David.Ruble@fdetrck-ccmail.army.mil.

Sincerely,

David L. Ruble, DVM
Major, U.S. Army
Chief, Animal Care & Use Review Division

Enclosure



United States Department of Agriculture
Animal and Plant Health Inspection Service
Animal Care

INSPECTION REPORT

Site 1

Louisiana State University
L.S.U. School of Veterinary Medicine
Baton Rouge, La. 70803

72-R-003 Site # 1

8-27-98

1000

Cindy -
No specific comments about
DCB. Lynn says that's how
they do it now - No new
is good news. DGB

NARRATIVE

panied me on this inspection.

previously identified that have been corrected.
3.1(a)- Kennels have been repaired.

your copy

... (s) identified this inspection
... (c) (1) - Surfaces of housing facilities must be constructed in
manner and made of materials that allow them to be cleaned and sanitized or
removed or replaced when worn or soiled.

Concrete floors in animal rooms and halls have cracked areas which need repair and
sealing. 48 cats, 10 Guinea pigs and 59 rabbits affected.

To be corrected by: 9-28-98

Section 3.6 (b)4- Resting Surfaces- No elevated resting surfaces provided for 29 cats
in Life Sciences Animal Care Facility- Mouse enclosures presently used as surfaces are
on cage floor and severely cut into available floor space. Elevated surfaces to be
provided by 9-28-98

Section 3.53- (c)- 2- Space Requirements- 2 Enclosures housing 4 rabbits each
measure 5 sq. Feet each. Rabbits weigh about 3 lbs. Each and thus require a total of 6
sq. Feet / cage.

To be corrected by: 9-1-98

This inspection consists of Previous sites (3)- School of Veterinary Medicine, (4)-
Pennington Biomedical Research, and (15)- Life Sciences animal Care facility

Prepared By:

Lynn P. Bourgeois

Title:

Lynn P. Bourgeois, Veterinary Medical Officer, USDA, APHIS, Animal Care

Date:

8/27/98

LARIS ID NO. 4002

Copy Received By:

R. A. Thomas

Title:

Assistant Director

Date:

8/27/98

From: Ruth Harris
To: "stobias@NATICK-CCMAIL.ARMY.MIL"@Domain.INET
Date: Fri, Nov 13, 1998 2:02 PM
Subject: Re: Re[2]: Annual Report to Congress

Steve

Here are the numbers:

We used 570 rats and 239 mice during the period 10/1/97 and 9/30/98. Of these 90 rats and 36 mice were used under the old grant DAMD 17-92-V-2009 (Military Nutrition Research: Six tasks to address medical factors limiting soldiers effectiveness) and 203 mice and 480 rats were used under the new grant DAMD 17-97-2-7013 (Military Nutrition Studies at the Pennington Biomedical Research Center).

As they were used for stress studies, most were categorized as Class C - drug intervention for pain or distress would interfere with the protocol.

I have attached a word document that describes our publications for the same 12 month time interval. Please let me know if this does not transmitt and I will FAX you the publication list. Also, let me know if you need more information.

Thanks
Ruth

CC: Ryan, Donna



United States Department of Agriculture
Animal and Plant Health Inspection Service
Animal Care

INSPECTION REPORT

Louisiana State University System
3810 West Lakeshore Drive
Baton Rouge, La. 70808

Site 1
Louisiana State University
L.S.U. School of Veterinary Medicine
Baton Rouge, La. 70803

72-R-003 Site # 1
8-27-98
1000

NARRATIVE

Assistant Director Rick Ramsey accompanied me on this inspection.

CATEGORY I: Non-compliant item(s) previously identified that have been corrected.
Structure & Construction- Section 3.1(a)- Kennels have been repaired.

CATEGORY III: Non-compliant item(s) identified this inspection

Section 3.1(c)(1) - Surfaces of housing facilities must be constructed in a manner and made of materials that allow them to be cleaned and sanitized or removed or replaced when worn or soiled.

Concrete floors in animal rooms and halls have cracked areas which need repair and sealing. 48 cats, 10 Guinea pigs and 59 rabbits affected.

To be corrected by: 9-28-98

Section 3.6 (b)4- Resting Surfaces- No elevated resting surfaces provided for 29 cats in Life Sciences Animal Care Facility- Mouse enclosures presently used as surfaces are on cage floor and severely cut into available floor space. Elevated surfaces to be provided by 9-28-98

Section 3.53- (c)- 2- Space Requirements- 2 Enclosures housing 4 rabbits each measure 5 sq. Feet each. Rabbits weigh about 3 lbs. Each and thus require a total of 6 sq. Feet / cage.

To be corrected by: 9-1-98

This inspection consists of Previous sites (3)- School of Veterinary Medicine, (4)- Pennington Biomedical Research, and (15)- Life Sciences animal Care facility

Prepared By: Lynn P. Bourgeois
Title: Lynn Bourgeois, Veterinary Medical Officer, USDA, APHIS, Animal Care

Date: 8/27/98
LARIS ID NO. 4002

Copy Received By:
Title:

R. A. Ramsey
Assistant Director

Date: 8/27/98

U.S. Army Medical Research and Materiel Command Animal Use Report

Facility Name: Pennington Biomedical Research Ctr. Principal Investigator: Donna H. Ryan
 Address: 6400 Perkins Road
Baton Rouge, LA 70808 Principal Investigator: Donna H. Ryan, M.D.
 (Typed/Printed Name)

Contract Number: DAMD 17-92-V-2009

This Report is for Fiscal Year (01 October - 30 September): 1998

AAALAC* Accreditation Status (circle one): Full Provisional Not Accredited

Date of Last USDA Inspection: 8/27/98 USDA Registration Number: 72-R-003 Site #1

Definitions of Column Headings on Back of Form					
A. Animal	B. Number of animals purchased, bred, or housed but not yet used	C. Number of animals used involving no pain or distress	D. Number of animals used in which appropriate anesthetic, analgesic, or tranquilizing drugs were used to alleviate pain	E. Number of animals used in which pain or distress was not alleviated	F. Total Number of Animals (Columns C+D+E)
Dogs					
Cats					
Guinea Pigs					
Hamsters					
Rabbits					
Non-human Primates					
Sheep					
Pigs					
Goats					
Horses					
Mice				36	36
Rats				90	90
Fish					
List Others:					

*AAALAC - Association for the Assessment and Accreditation of Laboratory Animal Care

U.S. Army Medical Research and Materiel Command Animal Use Report

Facility Name: Pennington Biomedical Research Ctr. Principal Investigator: Donna H. Ryan
(Signature)
 Address: 6400 Perkins Road
Baton Rouge, LA 70808 Principal Investigator: Donna H. Ryan, M.D.
(Typed/Printed Name)

Contract Number: DAMD 17-97-2-7013

This Report is for Fiscal Year (01 October - 30 September): 1998

AAALAC* Accreditation Status (circle one): Full Provisional Not Accredited

Date of Last USDA Inspection: 8/27/98 USDA Registration Number: 72-5-003 Site #1

Definitions of Column Headings on Back of Form

A. Animal	B. Number of animals purchased, bred, or housed but not yet used	C. Number of animals used involving no pain or distress	D. Number of animals used in which appropriate anesthetic, analgesic, or tranquilizing drugs were used to alleviate pain	E. Number of animals used in which pain or distress was not alleviated	F. Total Number of Animals (Columns C+D+E)
Dogs					
Cats					
Guinea Pigs					
Hamsters					
Rabbits					
Non-human Primates					
Sheep					
Pigs					
Goats					
Horses					
Mice				203	203
Rats				480	480
Fish					
List Others:					

*AAALAC - Association for the Assessment and Accreditation of Laboratory Animal Care



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
820 CHANDLER STREET
FORT DETRICK, MARYLAND 21702-5014

cc: Dr. Ryan
John
Ralph

REPLY TO
ATTENTION OF:

March 30, 1999

Special Projects Branch/SR/ljm

SUBJECT: Grant No. DAMD17-97-2-7013
Modification No. P90006

Mr. Patrick Marquette
Louisiana State University
Pennington Biomedical Research Center
6400 Perkins Road
Baton Rouge, Louisiana 70808

Dear Mr. Marquette:

Enclosed are fully executed copies of the above
subject modification for your records and the Principal
Investigator's.


If you have any questions concerning this matter,
you may contact Sherry Regalado, Contract Specialist,
at (301) 619-2376.

Sincerely,

Linda J. Mandeville
Procurement Technician

Enclosures

ASSISTANCE AGREEMENT

AWARD TYPE: <input type="checkbox"/> GRANT (31 USC 6304) <input checked="" type="checkbox"/> COOPERATIVE AGREEMENT (31 USC 6305) <input type="checkbox"/> OTHER TRANSACTION (10 USC 2371)			
AWARD NO: DAMD17-97-2-7013 Modification P90006		EFFECTIVE DATE See Grants Officer Signature Date Below	
		AWARD AMOUNT \$17,738,364.00	
		Page 1 of 1 Sherry Regalado (301) 619-2376	
PROJECT TITLE: Military Nutrition Research			
CFDA 12.420			
PERFORMANCE PERIOD: 1 July 1997 - 31 March 2000		PRINCIPAL INVESTIGATOR: Dr. Donna Ryan	
AWARDED AND ADMINISTERED BY: U.S. Army Medical Research Acquisition Activity ATTN: MCMR-AAA-v 820 Chandler St. Fort Detrick Maryland 21702-5014		PAYMENTS WILL BE MADE BY: EFT:T Army Vendor Pay DFAS-SA/FPAC 500 McCullough Ave. San Antonio, TX 78215-2100	
AWARDED TO: Louisiana State University Pennington Biomedical Research Center 6400 Perkins Road Baton Rouge, LA 70808		REMIT PAYMENT TO: Same as awarded to	
ACCOUNTING AND APPROPRIATION DATA: See Schedule			
SCOPE OF WORK: Accounting and Appropriation Data: 219204000009748119611102S15V0415000FABDODAMD179727013FABD00S18064 \$3,178,131 219204000009748119622787878V0415000FEXNODAMD179727013FEXN00S18064 \$ 362,000 219204000009748119622787879V0415000FHBAODAMD179727013FHBA00S18064 \$ 8,000			
1. The purpose of this modification is to add incremental funding to this Cooperative Agreement. As a result, Article 15, paragraph a. is replaced with the following: a. It is estimated that the total cost to the Government for the full performance of this cooperative agreement for the period 1 July 1997 - 31 March 2002 (research to be completed by 29 Feb 2002) shall be \$17,738,364.00. There have been funds allotted for payment of allowable costs incurred in the performance of this cooperative agreement in the amount of \$9,140,131. It is estimated that such funded amount shall be sufficient to cover allowable expenses for the period of 1 July 1997 - 31 March 2000. Subject to the availability of funds, it is estimated that additional funds will be provided by modification in accordance with the following incremental schedule: \$3,926,051 On or about 1 April 2000 \$4,094,617 On or about 1 April 2001 2. All other terms and conditions from the basic agreement remain the same.			
RECIPIENT ACCEPTED BY: Unilateral - does not require grantee signature _____ SIGNATURE		GRANTS OFFICER UNITED STATES OF AMERICA  SIGNATURE	
NAME AND TITLE 	DATE 	NAME AND TITLE B.C. BAKER III GRANTS OFFICER	DATE 3/29/99

ARMY PERSONNEL VISIT

Dr. Harris Lieberman, Chief, Military Nutrition Division*

Major Vicky Thomas, Nutritional Staff Officer (OSTG)**

Colonel Richard Lynch, Chief Dietician of the Army**

Colonel L. Sue Standage, Chief of Army Medical Specialist Corp**

Captain Mark Kellogg, Biochemist*

Captain Mary Chamberlain, USAF, Master Student, University of Southern Mississippi

Monday, February 22, 1999

8:00 Visitors: Tour of Center with Tulley and Rood

8:00 Lieberman: Ryan

9:00 Visitors: Tulley and Rood

9:30 Visitors: Harris

10:00 Visitors: DeLany

10:30 Visitors: Zachwieja

11:00 Visitors: Horohov

11:30 Visitors: Champagne

12:00 Lunch – Visitors, Lieberman, Ryan, Cline, Patrick, Champagne

1:00 Visitors: Cline

2:30 Lieberman: Ryan, Cline, DeLany, Zachwieja, Champagne, Phillips to discuss upcoming military conference

2:30 Visitors: Harsha and Kennedy to discuss PREMIER project

3:15 *Chiu Paratukul*

4:00 Lieberman: Harris and lab members

6:30 Dinner with remaining visitors – Ryan, Cline, DeLany, Harris

*Staying at the Hampton Inn (Constitution)

**Staying at the Quality Inn (Bluebonnet)

Page 2

Tuesday, February 23, 1999

8:00 Lieberman: Smith, Zachwieja, Horohov

8:00 Kellogg: DeLany

9:00 Kellogg and Lieberman: Horohov

10:00 Lieberman: Phillips to discuss publication of previous military conference

10:00 Kellogg: Tulley

11:00 Lieberman: Ryan Exit



USAMRMC Military Operational Medicine Research Program

1999 MOMRP Fact Sheet
Number 4



The Pennington Biomedical Research Center (PBRC) has gained national prominence in nutrition research since its origin in the early 1980s when a mutually beneficial research cooperation was established with a new Army biomedical nutrition research initiative. Research conducted by PBRC in collaboration with the U.S. Army Research Institute of Environmental Medicine (USARIEM) has led to new DoD nutrition guidelines and policies such as nutritional requirements in adverse operational environments, caloric and macronutrient standards for field rations, use of performance-enhancing ration supplements, permissible duration for feeding operational rations, appropriate levels of Basic Daily Food Allowance for specialized units, and nutritional requirements of military women.

Background

A series of specially funded cooperative agreements between PBRC and the U.S. Army Research and Material Command (USAMRMC) has provided high-quality analytical laboratory, nutrition database, and metabolic unit support for DoD nutrition-related research programs. The program currently supports the RDT&E-funded Military Nutrition research programs at the U.S. Army Soldier Systems Center (Natick) and USARIEM laboratories, as well as the Ration Sustainment Testing program. PBRC personnel frequently travel to DoD field studies to collect samples, returning to PBRC for laboratory analyses. Additionally, PBRC conducts research that complements and extends USARIEM's intramural program in areas of nutritional neuroscience, stress, physical, and mental performance, immune function, and garrison feeding. The PBRC program is periodically peer-reviewed by an external panel from the Committee on Military Nutrition Research (CMNR), Institute of Medicine (1988, 1990, 1996). This effort has led to significant improvements of operational rations, better understanding of warfighter energy and nutritional requirements, and modifications in garrison feeding.

Recent Goals and Accomplishments

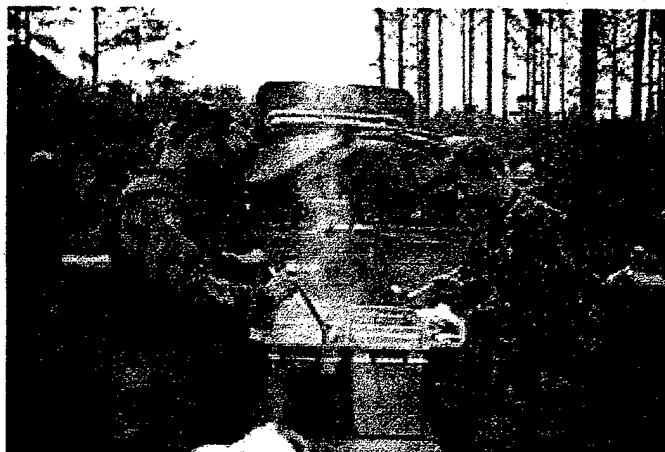
Stable Isotope Laboratory

Heavy atoms (non radioactive) are used as tracers to measure energy expenditure and changes in body water in free-ranging soldiers, marines, and sailors during field training in extreme climates. The lab showed that soldiers at work expend 4,000 kcal, even though their energy intakes are typically 75 percent or less of this requirement. In more extreme specialized training, the lab documented that energy expenditures may be as high as 8,000 kcal/24 hrs. in men and 4,800 kcal/24 hrs in women. Measurements of energy expenditure in studies in Alaska confirmed that the Military RDA for energy was adequate under these cold weather conditions. A field study in a desert environment showed that consumption of a carbohydrate supplemental drink significantly increased energy intake and reduced an energy imbal-

ance. Rangers were shown to expend more energy in hot, humid field environments than their assumed energy requirements. This lab has also been involved in studies on the limits of human endurance, including Norwegian Ranger studies, the Trans-Greenland Expedition, and Marine field exercises. Measurements of water intake using deuterium labeled water demonstrated that hydration was adequate in airmen consuming a new survival ration during a five-day survival exercise. Body water changes were also measured in several high altitude studies in relation to acute mountain sickness, with one study showing that hydration status decreased within one day at altitude, and that the effect was retained for at least six days after return to sea level.

Clinical Chemistry Laboratory for Human and Food Samples

This lab supports USARIEM and PBRC research projects with testing, field data collection, and methods development. For military studies, the lab developed a nutritional assessment panel which includes metabolic markers, vitamin E, vitamin A, vitamin C, enzymatic markers of B vitamins, folate, vitamin B12, prealbumin, retinol binding proteins, and other markers. The lab has adapted

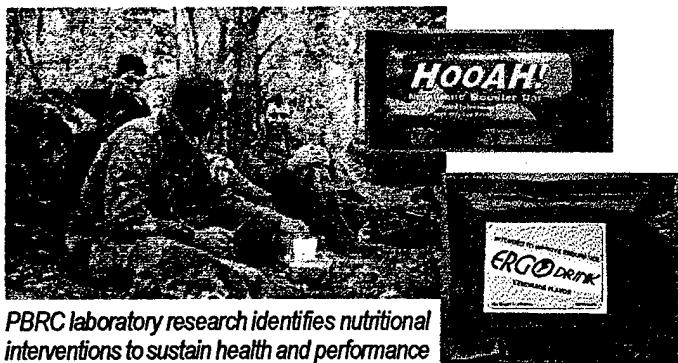


PBRC and USARIEM collaborative research has led to significant DoD nutrition guidelines and policies

methods for automation for salivary, urine, and plasma caffeine, salivary melatonin, glycerol, free fatty acids, lactate, ammonia, selenium, bromide, amino acids, retinol binding protein, fecal polyethylene glycol, urinary PABA and PAH, glutathione peroxidase, and countless immuno assays. A rapid, accurate, and easily automated method for analysis of vitamin C in blood, pharmaceuticals, and food was developed and patented. Also developed was a method for the analysis of nitrogen, calcium, magnesium, phosphorus, sodium, and potassium in food, feces, and urine by chemiluminescence and inductively coupled plasma emission spectrometry. Another original method was an ELISA method for measuring genotypes of fatty acid binding protein.

Stress, Nutrition, and Mental Performance

A multidisciplinary team, working to identify nutritional interventions for stress-induced behavior. The lab uses sleep deprivation and restraint models to identify the basic mechanisms that mediate stress-induced decrements in behavior. The lab discovered that ApoE is a factor that determines stress-responsiveness in mice. ApoE has the protective effect of repairing neuronal dam-



PBRC laboratory research identifies nutritional interventions to sustain health and performance in operational environments

age. This information could be useful to identify people with special susceptibilities to the negative aspects of stress. The lab is searching for additional markers of stress responsiveness. It is also developing an animal model that mimics human trials of exhaustive physical exercise to determine the optimal timing and composition of glucose and fat supplements to improve physical and cognitive performance.

Nutrition, Stress and Work Performance

A study on Special Operations Forces conducted at the PBRC demonstrated that chronic energy restriction in active young men and women decreases physical performance levels. A current study is evaluating creatine monohydrate supplementation to improve swimming power. The lab also developed new methodologies, including a skill-oriented task to assess anaerobic power and reaction time "on the feet" and a process to isolate myosin heavy chain protein from human skeletal muscle and to measure its synthesis rate in living subjects.

Nutrient Database Integration Laboratory

This lab analyzes food intake for military nutrition studies. For example, the lab supported the Savannah Ranger Study, El Paso Sergeants Major Academy Study, Special Forces Creatine Study, Fort Lewis Ranger Study, and a Marine field study of T-rations. PBRC programmers are streamlining the delivery of dietary intake information by setting up a custom-designed MiDAS System for USARIEM.

Enhancing Military Diets

This project produced over 60 modified recipes developed to meet Army needs and are now included in the master recipe file. These recipes serve 100 and provide lower fat and sodium intake, incorporate ethnic preferences, accommodate vegetarian diets, and expand breakfast choices. A survey of 2,000 military members from nine bases and representing all services determined current eating trends. In consultation with the Armed Forces Recipe Services and Armed Forces Food Policy Council, PBRC devises strategies for improved garrison intake using behavior modification techniques. The PBRC designed and implemented a model health promotion program for military service personnel and their families at Fort Polk. Nutritional assessments were conducted on 200 military wives to establish routine eating, activity, and other behavioral patterns. Cardiovascular risk assessments of 125 military families were also performed. Based on this information, a program for family health promotion, including prudent eating, exercise, stress and smoking reduction was developed and implemented on about 70 intact military families. A manual providing instructions for developing such a program was produced.

Stress, Nutrition, and Immune Function Laboratory

This lab evaluates stress-induced immune modulation in animals and humans. A rodent sleep deprivation model for stress-induced changes in immune function was developed. Using this model, the impact of dietary fat acids on immune responses and stress-induced immune function was assessed. The lab demonstrated that animals deprived of sleep and fed a high-fat diet suffer from impaired immune function, while animals deprived of sleep but fed a low-fat diet have normal immune function. This can be further explored through clinical investigations.

Metabolic Unit Project

Studies through this project included demonstrating the benefits of testosterone and alendronate to prevent the loss of bone and muscle mass caused by a novel model of weightlessness through collaboration with NASA. A trial conducted in the Center's sleep lab provided conclusive evidence of the relative benefits for alertness and performance of caffeine, tyrosine, phentermine and amphetamine during sleep deprivation. Additionally, the study documented the negative effects on recovery sleep produced by amphetamine. These findings benefit policy development for sustained operations for warfighters.

*For more information on research projects and updates at the
Pennington Biomedical Research Center
visit the web site below.*

<http://www.pbrc.edu>



Army Correspondence file
Copy to Dr. Ryan
Underwood
Farrell



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
820 CHANDLER STREET
FORT DETRICK, MARYLAND 21702-5014

REPLY TO
ATTENTION OF:

May 14, 1999

Special Projects Branch

SUBJECT: Cooperative Agreement No. DAMD17-97-2-7013

Mr. Patrick Marquette
Louisiana State University
Pennington Biomedical Research Center
Baton Rouge, Louisiana 70808

Dear Mr. Marquette:

This letter serves as notification of a shortfall in available funding for subject five-year effort involving eight (8) tasks to address medical factors limiting soldier effectiveness.

A recent review of this agreement revealed that for Year 2 (ended June 1999), congressional funding available was \$452,777 below your estimated Year 2 budget. As well, Year 3 funding for July 1999 through June 2000 will be short by \$124,788 when compared to the estimated Year 3 budget.

Therefore, it is requested that you submit a revised budget as soon as possible to be reflective of Year 2 and Year 3's decrease in their respective total estimated budgets. It is suggested that the decrement be implemented proportionally across all funded tasks.

After review of your revised budgets, the cooperative agreement will be modified to incorporate the new estimated budget and revise the payment schedule.

Please provide the revised budget to this office by 28 May 1999.

If you have any questions, please call Ms. Sherry Regalado, the Contract Specialist recently assigned contract administration for this agreement. Her telephone number is (301) 619-2376.

Sincerely,

Michael A. Younkins
Michael A. Younkins
Contracting Officer

file
guar up

Dr. Donna Ryan
Associate Executive Director for Science
Pennington Biomedical Research Center
Baton Rouge, LA

July 1, 1999

Dear Dr. Ryan:

This letter is to inform you of my resignation, effective July 30, 1999. Family obligations are requiring my relocation to another state. I thank you for the opportunity I have had to work at such a fine institution as Pennington Biomedical Research Center, and appreciate the welcome and support I received from you and the staff here at PBRC. I will proudly remember my experiences here and will willingly tell my professional colleagues of the high quality scientific work being accomplished by the research faculty at PBRC.

If you deem it appropriate, I would like to maintain professional collaboration with the faculty and participate in any scientific endeavors that may benefit from my experience.

My experience here at PBRC has been very rewarding, and my departure comes after much introspection. Thank you, again, for your support.

Respectfully,

Alana D. Cline

Alana D. Cline
Assistant Professor - Research

cc/gork

APPENDIX II

CLINICAL LABORATORY FOR HUMAN AND FOOD SAMPLES

Best #'s

fecal nitrogen recovery

hcl digestion

sample	amt spike	mg/ml	weight	mg/g	recovered	spiked/g	% recovery
exp 1							
blank 1	0	0.01767	0.3423	5.162138			
blank 2	0	0.01786	0.3423	5.217645			
blank 3	0	0.01749	0.3423	5.109553			
ave blank				5.163112			
2	0.7	0.02415	0.3363	7.181088	2.017976	2.081475	96.94933
2.1	0.7	0.02404	0.32895	7.308102	2.144989	2.127983	100.7992
2.3	0.7	0.02426	0.32895	7.374981	2.211869	2.127983	103.942
2 ave							100.5635 100.5635
3	1.4	0.03341	0.3706	9.015111	3.851998	3.777658	101.9679
3.1	1.4	0.03322	0.3706	8.963842	3.80073	3.777658	100.6108
3.3	1.4	0.0336	0.3706	9.066379	3.903267	3.777658	103.325
3 ave							101.9679 101.9679
4	2.8	0.04703	0.3807	12.35356	7.190447	7.354873	97.7644
4.1	2.8	0.04688	0.3807	12.31416	7.151046	7.354873	97.22868
4.3	2.8	0.04717	0.3807	12.39033	7.227221	7.354873	98.2644
4 ave							97.75249 97.75249

sample	amt spike	mg/ml	weight	mg/g	recovered	spiked/g	% recovery
blank 1	0	0.01728	0.3332	5.186074			
blank 1.1	0	0.01757	0.3332	5.273109			
blank 1.3	0	0.01699	0.3332	5.09904			
blank 2	0	0.01655	0.3147	5.258977			
blank 2.1	0	0.01669	0.3147	5.303464			
blank 2.3	0	0.01641	0.3147	5.21449			
average		0.016915		5.222526			
5	4.2	0.06091	0.3929	15.50267	10.28015	10.68974	96.16833
5.1	4.2	0.06109	0.3929	15.54849	10.32596	10.68974	96.5969
5.3	4.2	0.06072	0.3929	15.45431	10.23179	10.68974	95.71594
ave 5							96.16039 96.16039
6	4.2	0.06448	0.3457	18.65201	13.42948	12.14926	110.5374
6.1	4.2	0.06452	0.3457	18.66358	13.44106	12.14926	110.6327
6.2	4.2	0.06444	0.3457	18.64044	13.41791	12.14926	110.4422
ave 6		0.062693					110.5374 110.5374
overall ave							101.3963

ANTEK-9000 Calibration Report 7/1/99

Cal Name: First.cal

Cal Checked: 12/31/03
7:00 PM

Method Name: first.mth

Blank Correction is OFF

*Recovery
(Blank \pm 0.42 mg/mL)*

Ck Std % Error

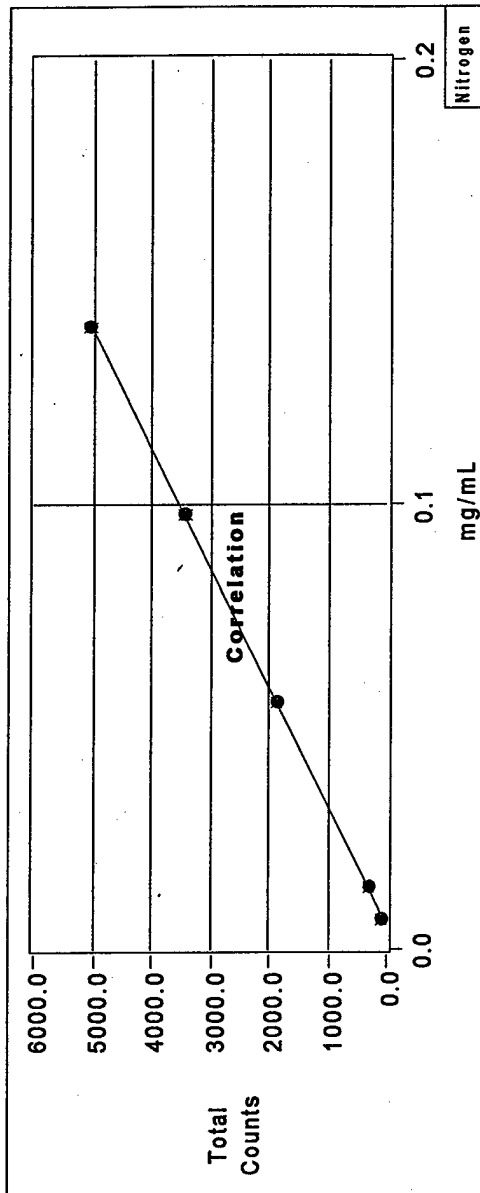
0.00

Cal Order

1

Correlation

0.9999



Name	Use A	AConc	ACnts	%RSDcnts	Time	Date	OpID
Standard 1	X	0.00700	80.74	10.28			
Standard 1.1	X	0.00700	86.61		9:24 AM	7/1/99	SWS
Standard 1.2	X	0.00700	74.86		9:28 AM	7/1/99	SWS
Standard 2	X	0.01400	297.96	0.29			
Standard 2.2	X	0.01400	297.34		9:39 AM	7/1/99	SWS
Standard 2.3	X	0.01400	298.57		9:43 AM	7/1/99	SWS
Standard 3	X	0.05600	1863.51	0.84			
Standard 3.2	X	0.05600	1852.43		9:50 AM	7/1/99	SWS
Standard 3.3	X	0.05600	1874.59		9:53 AM	7/1/99	SWS
Standard 4	X	0.09800	3431.13	1.40			
Standard 4.1	X	0.09800	3465.18		9:57 AM	7/1/99	SWS
Standard 4.2	X	0.09800	3397.09		10:00 AM	7/1/99	SWS
Standard 5	X	0.14000	5058.86	0.63			

METHOD COMPARISON INFORMATION SHEET

Attach a printout of the XY data to this form.

If possible, send data electronically (spreadsheet, word processor, ASCII).

Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE:	10/15/98
ANALYTE:	Homocysteine
UNITS:	umol/L
Imx	Abbott Imx
LOT #	rgt = 43836M300
CONTROLS (CON6,ECS,etc):	Abbott Imx
RUN BIAS (in terms of SD's):	
ASSAY DATE:	10/15/98
COMPETITIVE KIT:	
LOT # IF AVAILABLE:	
RUN BIAS (in terms of SD's):	
ASSAY DATE:	
SITE OF THE STUDY:	Pennington Biomedical Research
FRESH OR FROZEN SPECIMENS:	
ANTICOAGULANTS, SST, etc.:	

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

Approved - ~~10/15/98~~
11-4-98
Jennifer Reed

Minimum Detection Limit Calculation

Instrument: IMx Serial #17545

Test: Homocysteine

0 (A) Calibrator rate found

1	201.27
2	201.47
3	201.31
4	202.06
5	202.29
6	201.74
7	201.72
8	201.94
9	202.14
10	202.2

Mean 201.814

SD 0.37131

Mean - 2SD 201.07138

bottle conc found rate

0 201.814

2.5 187.155

m -5.8636

b 201.814

min det lim 0.1266492

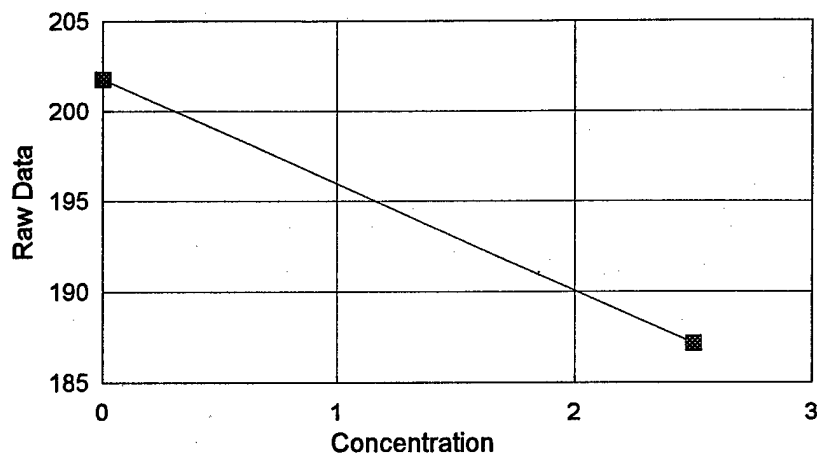
Bottle

Conc of B: 2.5

rate 1 187.14

rate 2 187.17

Mean of B 187.155 rate



Package Sens = $< 0.50 \mu\text{mol/L}$

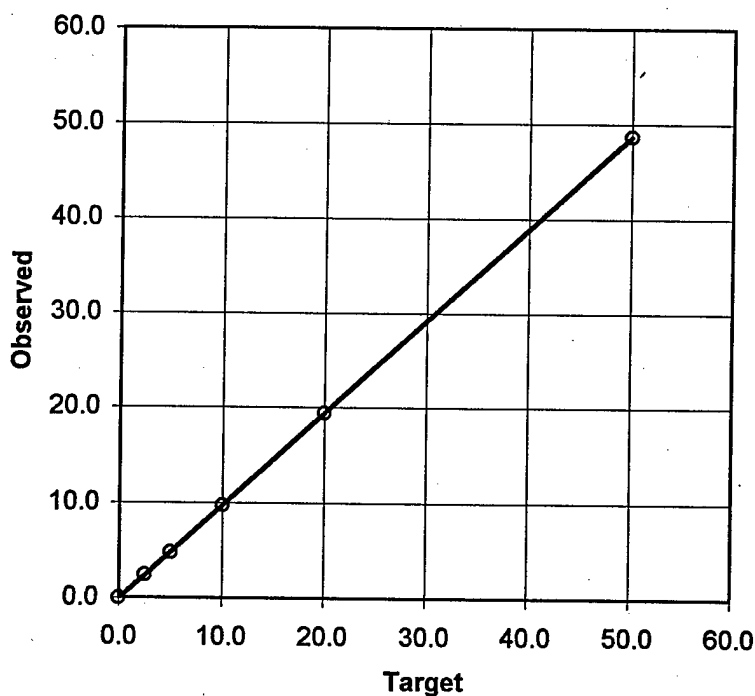
IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	Homocysteine	CDC Test System ID Code:	
Dose Unit:	umol/L	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Joanie B. Wilson

Imx Homocysteine Calibration Evaluation - Calibrator Lot # 43839M300

Calibrator	Target Conc.	Acceptable Limits Lower Upper	Observed Conc.	Within Range	O/T
A	0.0		0.0	Yes	100.0%
B	2.5		2.4		96.4%
C	5.0		4.8		95.2%
D	10.0		9.7		97.3%
E	20		19.38		96.9%
F	50		48.66		97.3%

Average:	97.2%
----------	-------



Date(s) Performed: 10/15/98
Performance Specification:

Within Run Precision

TEST	Date	umol/L		
		5.25-8.75	10.0-15.0	20.0-30.0
		Homocysteine		
		Low	Medium	High
	10/15/1998	7.2	12.76	26.34
		7.13	12.63	25.9
		6.92	12.67	25.85
		6.92	12.62	25.94
		6.93	12.53	25.48
		7.09	12.85	25.66
		7.05	12.73	25.86
		7.13	12.65	25.58
		7.1	12.63	25.45
		7.06	12.54	25.55
Mean		7.1	12.7	25.8
SD		0.09	0.09	0.26
CV		1.33	0.73	1.00
Package		5.9	10.8	21.6
SD		0.13	0.21	0.31
CV		2.2	1.9	1.4

Lab 307362

UNITY-PC Summary Data Report

Lot 43842
Abbott Homocysteine

Data for October 1998

Printed 4 November 1998 / Page 1

Analyte Method Instrument/Kit Reagent Unit Temperature	Level 1		Level 2		Level 3	
	Month	Cumulative	Month	Cumulative	Month	Cumulative
Homocysteine						
FPIA	Mean	7.14	7.14	12.70	12.70	25.93
Abbott IMx	SD	0.14	0.14	0.10	0.10	0.53
Dedicated Reagent	CV	1.9 ✓	1.9	0.8 ✓	0.8	2.1 ✓
μmol/L	# Points	7	7	7	7	7
	Fixed Mean	7.00	12.50	25.00		
	Fixed SD	0.88	1.25	2.50		

Package

\bar{x}	5.9	10.8	21.6
SD	0.30	0.44	0.81
CV	5.2	4.1	3.7

INFORMATION SHEET

Attach a printout of the XY data to this form.
If possible, send data electronically (spreadsheet, word processor, ASCII).
Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE:	12/1/98
ANALYTE:	C-Peptide
UNITS:	ng/mL
HPLC	Immulite
LOT #	
CONTROLS (CON6,ECS,etc):	Biorad
RUN BIAS (in terms of SD's):	
ASSAY DATE:	12/1/98
COMPETITIVE KIT:	
LOT # IF AVAILABLE:	
RUN BIAS (in terms of SD's):	
ASSAY DATE:	
SITE OF THE STUDY:	Pennington Biomedical Research
FRESH OR FROZEN SPECIMENS:	
ANTICOAGULANTS, SST, etc.:	

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

Approved: J. Ross, Ph.D.
12-2-98

IMMULITE METHOD EVALUATION

Lab ID: Pennington Biomedical Res Ctr
 Analyte: CPE
 Dose Unit: ng/mL
 Lab Director: Richard Tulley, Ph.D.

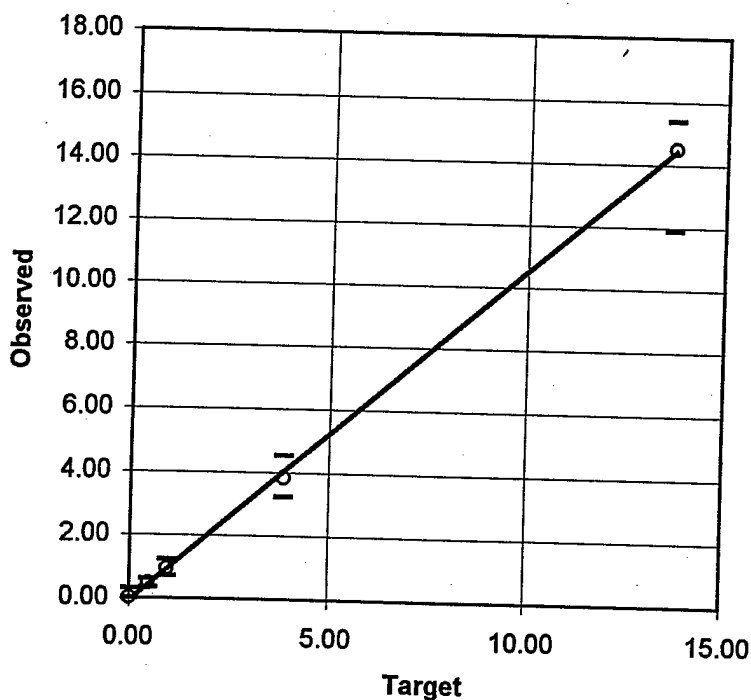
CLIA Complexity: Moderate
 CDC Test System ID Code:
 CDC Analyte ID Code
 Lab Technologist: Janani Prabakaran

IMMULITE CPE Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits Lower	Acceptable Limits Upper	Observed Conc.	Within Range	O/T
0	0.00	0.00	0.30	0.03	Yes	100.0%
1	0.48	0.35	0.61	0.48	Yes	100.0%
2	0.96	0.70	1.22	0.95	Yes	99.0%
3	3.88	3.24	4.52	3.84	Yes	99.0%
4	13.70	11.90	15.40	14.50	Yes	105.8%

Average:

100.8%



Date(s) Performed: 11/30/98
 Performance Specification:

Minimum Detection Limit Calculation

Instrument: IMMULITE
Test: C-PEPTIDE

0 (A) Calibrator rate found

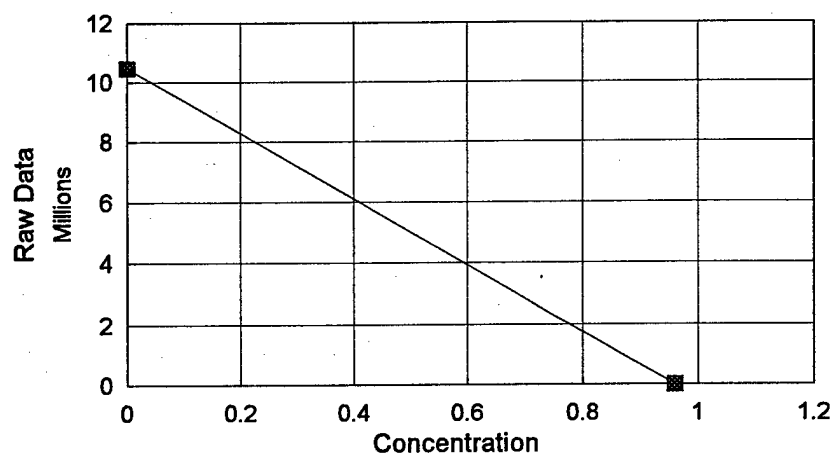
1	10503350
2	10312145
3	9771200
4	10902918
5	10357978
6	10837224
7	10623573
8	10656596
9	10563285
10	10035855

Mean 10456412
SD 350065.51
Mean - 2SD 9756281.4

bottle conc	found rate
0	10456412
0.96	1.02

m -10892095
b 10456412
min det lim 0.0642788

Bottle
Conc of B: 0.96
rate 1 0.84
rate 2 1.2
Mean of B 1.02 rate



Pennington Biomedical
 Clinical Research Lab
 DPC Immulite
 Within Run Precision

TEST	C-Peptide		
	Biorad1	Biorad2	Biorad3
	1.5	6.4	14.0
	1.5	6.4	14.6
	1.7	6.2	14.4
	1.7	6.8	12.4
	1.7	6.4	14.4
	1.5	6.1	13.2
	1.5	6.8	14.0
	1.9	7.0	13.0
	1.5	6.9	13.2
	1.8	6.2	13.4
Mean	1.63	6.52	13.66
SD	0.149443	0.325918	0.724492
CV	9.168307	4.998735	5.303749

Package 0.18 0.6 1.4
 SD
 CV 10.5 9.8 10.6
 Sens 0.3

Pennington Biomed Res Ctr
 Clinical Research Lab
 DPC Immulite
 Run-to-Run Precision ng/mL

1.34-2.06 5.0-7.4 10.4-16.0

TEST

Date

C-Peptide

	Biorad1	Biorad2	Biorad3
12/1/98	1.5	6.4	14.0
	1.5	6.4	14.6
	1.7	6.2	14.4
	1.7	6.8	12.4
	1.7	6.4	14.4
12/1/98	1.5	6.1	13.2
	1.5	6.8	14.0
	1.9	7.0	13.0
	1.5	6.9	13.2
	1.8	6.2	13.4

Mean

SD

CV

CV

1.63	6.52	13.66
0.149443	0.325918	0.724492
9.168307	4.998735	5.303749
6134.969	1533.742	732.0644

INFORMATION SHEET

Attach a printout of the XY data to this form.

If possible, send data electronically (spreadsheet, word processor, ASCII).

Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE: 5/18/99

ANALYTE: CRP

UNITS: mg/dl

Instrument: DPC Immulite

LOT #

CONTROLS (CON6,ECS,etc):

RUN BIAS (in terms of SD's):

ASSAY DATE: 5/12/99

COMPETITIVE KIT:

LOT # IF AVAILABLE:

RUN BIAS (in terms of SD's):

ASSAY DATE:

SITE OF THE STUDY: Pennington Biomedical Research

FRESH OR FROZEN SPECIMENS:

ANTICOAGULANTS, SST, etc.:

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

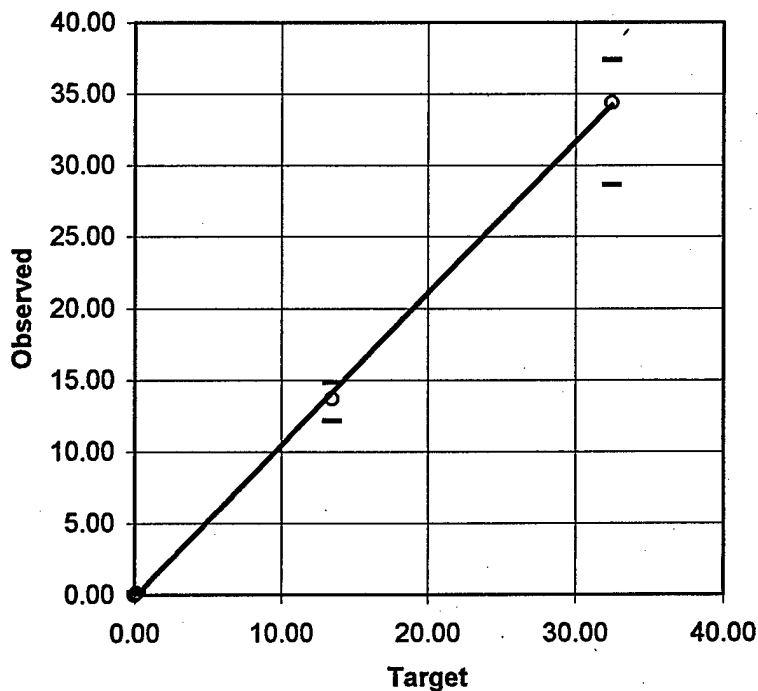
IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	CRP	CDC Test System ID Code:	
Dose Unit:	mg/dl	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE CRP Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
A	0.00			0.00	Yes	100.0%
D	0.13	0.09	0.17	0.13	Yes	100.0%
K	13.46	12.11	14.81	13.70	Yes	101.8%
L	32.48	28.61	37.35	34.40	Yes	105.9%

Average:	101.9%
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Date(s) Performed: 5/12/99
 Performance Specification:

IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomed Res Ctr	CLIA Complexity:	Moderate
Analyte:	CRP	CDC Test System ID Code:	
Dose Unit:	mg/dl	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE CRP Sensitivity Evaluation

Instrument: Immulite

Assay Type: S (Enter S for sandwich, C for competitive)

Replicates of the zero (CPS):

Four Parameters:

1	246997
2	234277
3	222335
4	238570
5	224851
6	231531
7	224566
8	236821
9	235380
10	218629
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	

P1	64700000
P2	203000
P3	23
P4	-1.12

Slope	1.014302
Intercept	-42752.07

Analytical Sensitivity

Sample	CPS	Dose #NUM!
1SD	240108	
2SD	248819	0.006
3SD	257531	0.013
4SD	266243	0.020

AVG	231396
SD	8712
CV	3.8%

Date(s) Performed:
Performance Specification:

Laboratory Criteria:

Acceptable: Y N

Reviewed & Approved by:
Signature/Date:

Janani Prabakaran, Ph.D.
6-15-99

**Pennington Biomedical
Clinical Research Lab
DPC Immulite
Within Run Precision**

TEST

CRP	
CRP 1	CRP 2
1.01	14.00
1.03	13.50
1.07	13.60
1.02	14.00
1.00	13.60
0.99	14.30
0.97	14.10
1.09	14.80
1.08	15.10
1.03	14.10
1.02	13.60
1.028182	14.06364
0.037899	0.51434
3.686059	3.657232

Package	0.99	13.9
SD	0.038	0.85
CV	3.8	6.2
Sens	0.01	
Units	mg/dl	

INFORMATION SHEET

Attach a printout of the XY data to this form.

If possible, send data electronically (spreadsheet, word processor, ASCII).

Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE:	5/18/99
ANALYTE:	Myoglobin
UNITS:	ng/ml
INSTRUMENT:	DPC Immulite
LOT #	
CONTROLS (CON6,ECS,etc):	Immulite Myoglobin
RUN BIAS (in terms of SD's):	
ASSAY DATE:	5/7/99
COMPETITIVE KIT:	
LOT # IF AVAILABLE:	
RUN BIAS (in terms of SD's):	
ASSAY DATE:	
SITE OF THE STUDY:	Pennington Biomedical Research
FRESH OR FROZEN SPECIMENS:	
ANTICOAGULANTS, SST, etc.:	

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

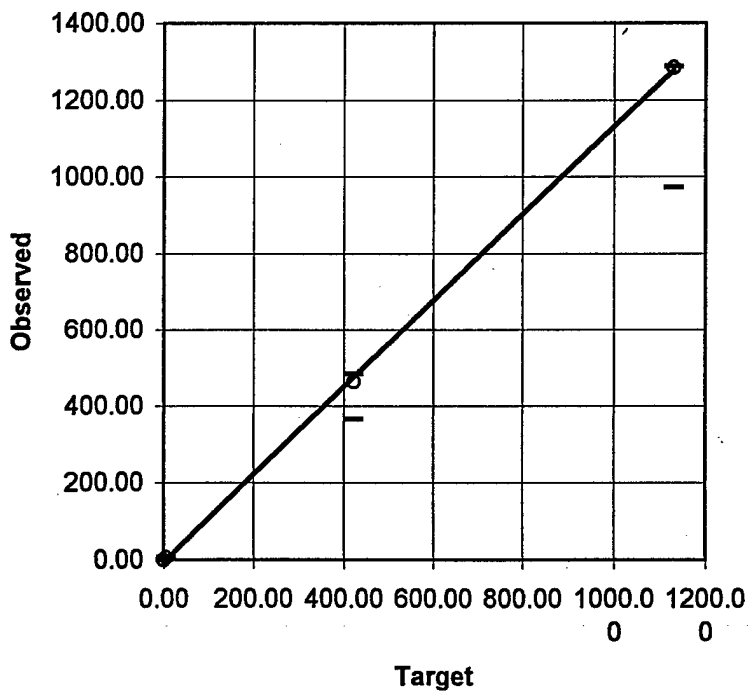
IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	myoglobin	CDC Test System ID Code:	
Dose Unit:	ng/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE MYOGLOBIN Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
0	0.00	0.00	0.50	0.01	Yes	100.0%
1	6.00	4.80	7.20	6.36	Yes	106.0%
2	423.00	364.00	482.00	461.60	Yes	109.1%
3	1130.00	972.00	1288.00	1284.00	Yes	113.6%

Average:	107.2%
----------	--------



Date(s) Performed: 5/19/99
Performance Specification:

IMMULITE METHOD EVALUATION

Lab ID: Pennington Biomed Res Ctr
Analyte: Myoglobin
Dose Unit: ng/mL
Lab Director: Richard Tulley, Ph.D.

CLIA Complexity: Moderate
CDC Test System ID Code:
CDC Analyte ID Code
Lab Technologist: Janani Prabakaran

IMMULITE MYOGLOBIN Sensitivity Evaluation

Instrument: Immulite

Assay Type: **S** (Enter S for sandwich, C for competitive)

Replicates of the zero (CPS):

1	695296
2	609475
3	622218
4	647496
5	646289
6	633208
7	685270
8	586104
9	647337
10	664295
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	

AVG 643699
SD 33181
CV 5.2%

Four Parameters:

P1	208000000
P2	718000
P3	756
P4	-0.989

Slope 1.072192
Intercept 64804.89

Analytical Sensitivity

Sample	CPS	Dose
1SD	676880	0.242
2SD	710061	0.363
3SD	743242	0.484
4SD	776424	0.605

Date(s) Performed:
Performance Specification:

Laboratory Criteria:

Acceptable: **Y** N

Reviewed & Approved by:
Signature/Date:

Janani Prabakaran, Ph.D.
6-15-99



Package	25.8	219
SD	0.66	7.0
CV	2.6	3.2
Sens	0.5	
Units	ng/ml	

INFORMATION SHEET

Attach a printout of the XY data to this form.
If possible, send data electronically (spreadsheet, word processor, ASCII).
Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE: 5/19/99
ANALYTE: IL-6
UNITS: pg/ml

Instrument: DPC Immulite
LOT #

CONTROLS (CON6,ECS,etc):
RUN BIAS (in terms of SD's):
ASSAY DATE: 5/19/99

COMPETITIVE KIT:
LOT # IF AVAILABLE:
RUN BIAS (in terms of SD's):
ASSAY DATE:

SITE OF THE STUDY: Pennington Biomedical Research

FRESH OR FROZEN SPECIMENS:
ANTICOAGULANTS, SST, etc.:

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

IMMULITE METHOD EVALUATION

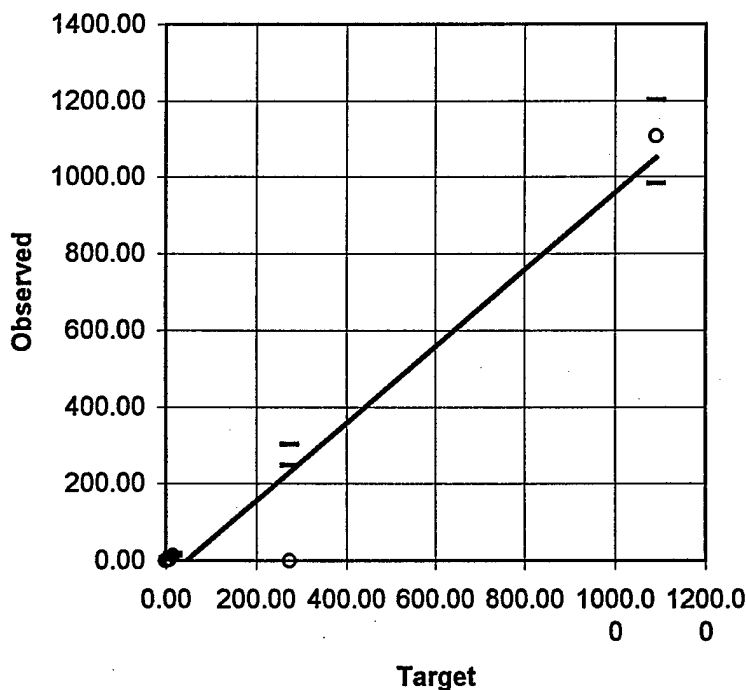
Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	IL-6	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE IL- 6 Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
A	0.00			0.04	Yes	100.0%
Cx3	5.13	4.36	5.9	2.075	No	40.4%
Cx2	7.69	6.54	8.85	3.85	No	50.1%
C	15.38	13.07	17.69	13.38	Yes	87.0%
G	273.87	246.50	301.30	262..50	Yes	#VALUE!
H	1091.73	982.30	1201.00	1106.50	Yes	101.4%

Average:

#VALUE!



IMMULITE METHOD EVALUATION

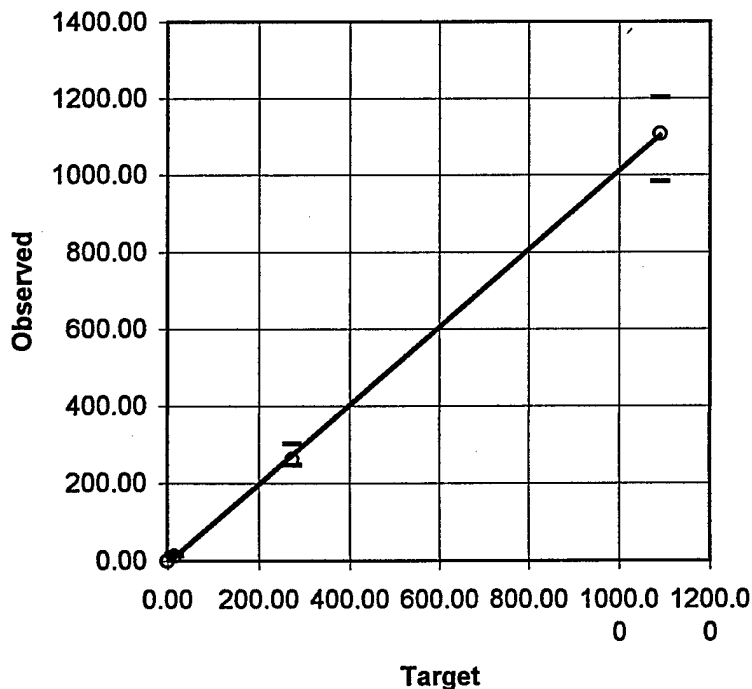
Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	IL-6	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE IL-6 Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
A	0.00			0.04	Yes	100.0%
C	15.38	13.07	17.69	13.38	Yes	87.0%
G	273.87	246.50	301.30	262.50	Yes	95.8%
H	1091.73	982.30	1201.00	1106.50	Yes	101.4%

✓
gmad

Average:	96.0%
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Date(s) Performed: 5/19/99
Performance Specification:

IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomed Res Ctr	CLIA Complexity:	Moderate
Analyte:	IL-6	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE IL-6 Sensitivity Evaluation

Instrument: Immulite

Assay Type: **S** (Enter S for sandwich, C for competitive)

Replicates of the zero (CPS):

Four Parameters:

1	389953
2	422941
3	425012
4	388814
5	402839
6	384292
7	413726
8	387086
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	

P1	152000000
P2	273000
P3	16400
P4	-0.952

Slope	0.7964342
Intercept	-63319.68

Analytical Sensitivity

Sample	CPS	Dose #NUM!
1SD	418571	0.691
2SD	435308	1.647
3SD	452046	2.633
4SD	468784	

AVG	401833
SD	16738
CV	4.2%

Date(s) Performed:
Performance Specification:

Laboratory Criteria:

Acceptable: **(Y)** N

Reviewed & Approved by:
Signature/Date:

Janani Prabakaran, Ph.D.
6-15-99

**Pennington Biomedical
Clinical Research Lab
DPC Immulite
Within Run Precision**

TEST	IL-6	
	Cyto 1	Cyto 2
	28.8	148.0
	24.1	161.0
	26.6	147.0
	21.2	146.0
	26.9	130.0
	24.3	158.0
	25.3	143.0
	26.4	138.0
	24.5	144.0
	25.8	150.0
Mean	25.39	146.5
SD	2.04855	8.947377
CV	8.068333	6.107425

Package	96	200
SD	5.3	8.2
CV	5.5	4.1
Sens	5	
Units	pg/ml	

INFORMATION SHEET

Attach a printout of the XY data to this form.
If possible, send data electronically (spreadsheet, word processor, ASCII).
Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE: 7/2/99
ANALYTE: IL-1B
UNITS: pg/ml

Instrument: DPC Immulite

LOT #

CONTROLS (CON6,ECS,etc):

RUN BIAS (in terms of SD's):

ASSAY DATE: 7/2/99

COMPETITIVE KIT:

LOT # IF AVAILABLE:

RUN BIAS (in terms of SD's):

ASSAY DATE:

SITE OF THE STUDY: Pennington Biomedical Research

FRESH OR FROZEN SPECIMENS:

ANTICOAGULANTS, SST, etc.:

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

IMMULITE METHOD EVALUATION

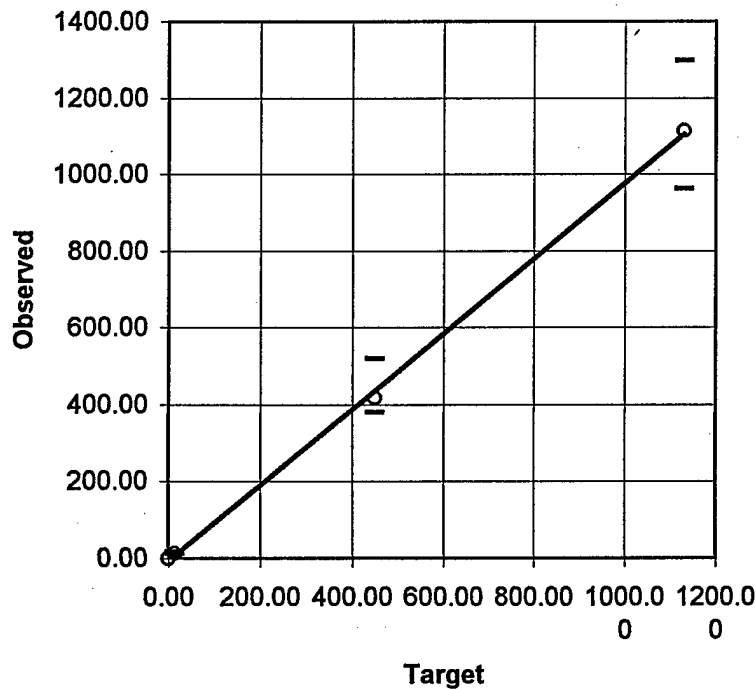
Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	II-1B	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE II-1B Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
		Lower	Upper			
A	0.00			0.17	Yes	100.0%
B	13.96	10.96	16.96	12.44	Yes	89.1%
G	449.09	380.09	518.09	416.80	Yes	92.8%
I	1130.00	962.00	1298.00	1112.60	Yes	98.5%

✓
gm

Average:	95.1%
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Date(s) Performed: 7/2/99
Performance Specification:

IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomed Res Ctr	CLIA Complexity:	Moderate
Analyte:	IL-1B	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE IL-1B Sensitivity Evaluation

Instrument: Immulite

Assay Type: **S** (Enter S for sandwich, C for competitive)

Replicates of the zero (CPS):

1	72193
2	56269
3	61515
4	81042
5	68129
6	68172
7	65436
8	66566
9	64463
10	71373
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	

AVG	67516
SD	6646
CV	9.8%

Four Parameters:

P1	261000000
P2	50700
P3	6320
P4	-1.02

Slope	0.8370363
Intercept	-1244.555

Analytical Sensitivity

Sample	CPS	Dose
1SD	74162	0.299
2SD	80809	0.460
3SD	87455	0.619
4SD	94101	0.778

Date(s) Performed:
Performance Specification:

Laboratory Criteria:

Acceptable: **Y** N

Reviewed & Approved by:
Signature/Date:

Janani Prabakaran, Ph.D.
7-21-99

TEST

IL-1B	
Cyto 1	Cyto 2
97.0	463.0
95.0	462.0
99.6	463.0
101.0	420.0
100.0	460.0
102.0	445.0
90.5	448.0
99.5	453.0
104.0	453.0
108.0	454.0
99.66	452.1
4.813223	12.91382
4.829643	2.856407

Mean

SD

CV

Package

SD

CV

Sens

Units

39

1.1

2.8

1.5

pg/ml

669

32

4.8



INFORMATION SHEET

Attach a printout of the XY data to this form.

If possible, send data electronically (spreadsheet, word processor, ASCII).

Provide as much relevant information as possible.

ESSENTIAL INFORMATION

DATE: 5/19/99
ANALYTE: TNA
UNITS: pg/ml
Instrument: DPC Immulite
LOT #
CONTROLS (CON6,ECS,etc):
RUN BIAS (in terms of SD's):
ASSAY DATE: 5/19/99
COMPETITIVE KIT:
LOT # IF AVAILABLE:
RUN BIAS (in terms of SD's):
ASSAY DATE:
SITE OF THE STUDY: Pennington Biomedical Research
FRESH OR FROZEN SPECIMENS:
ANTICOAGULANTS, SST, etc.:

OTHER OPTIONAL INFORMATION

(TEST POPULATION, SENIOR INVESTIGATOR, etc)

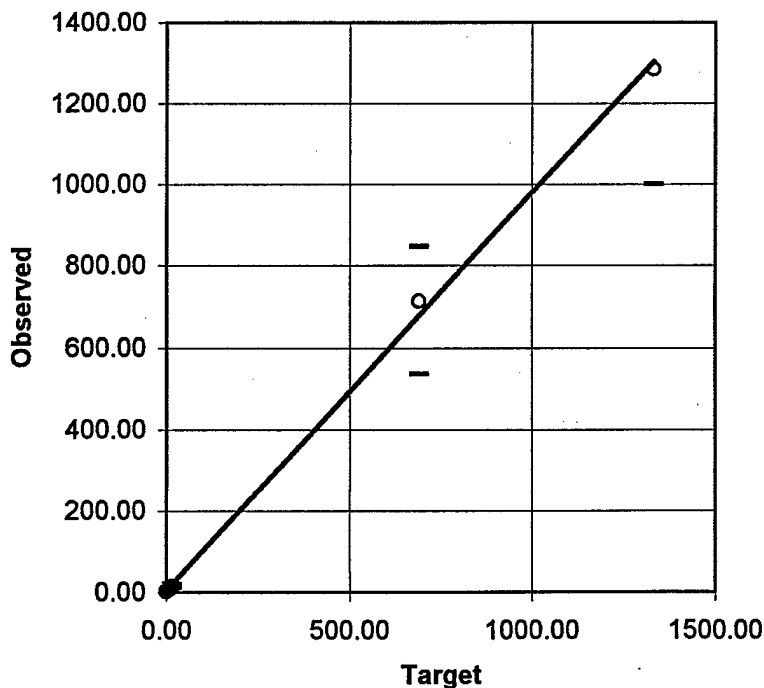
IMMULITE METHOD EVALUATION

Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	TNA	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE TNA Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
		Lower	Upper			
A	0.00			1.60	Yes	100.0%
Bx3	5.3	3.71	6.89	4.8	Yes	90.6%
Bx2	7.95	5.565	10.335	6.65	Yes	83.6%
B	15.90	11.13	20.67	16.30	Yes	102.5%
G	690.00	534.75	845.25	714.00	Yes	103.5%
H	1330.00	1000.00		1285.00	Yes	96.6%

Average:	96.1%
----------	-------



IMMULITE METHOD EVALUATION

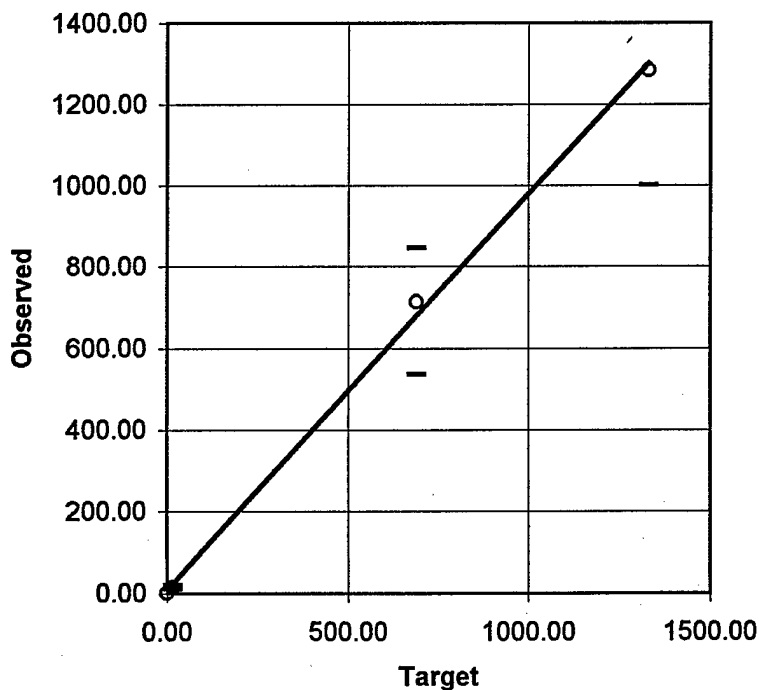
Lab ID:	Pennington Biomedical Res Ctr	CLIA Complexity:	Moderate
Analyte:	TNA	CDC Test System ID Code:	
Dose Unit:	pg/ml	CDC Analyte ID Code	
Lab Director:	Richard Tulley, Ph.D.	Lab Technologist:	Janani Prabakaran

IMMULITE TNA Calibration Evaluation - Calibrator Lot # 0001

Calibrator	Target Conc.	Acceptable Limits		Observed Conc.	Within Range	O/T
		Lower	Upper			
A	0.00			1.60	Yes	100.0%
B	15.90	11.13	20.67	16.30	Yes	102.5%
G	690.00	534.75	845.25	714.00	Yes	103.5%
H	1330.00	1000.00		1285.00		96.6%

✓
JP

Average:	100.7%
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Date(s) Performed: 5/19/99
Performance Specification:

IMMULITE METHOD EVALUATION

Lab ID: Pennington Biomed Res Ctr
Analyte: TNA
Dose Unit: pg/ml
Lab Director: Richard Tulley, Ph.D.

CLIA Complexity: Moderate
CDC Test System ID Code:
CDC Analyte ID Code
Lab Technologist: Janani Prabakaran

IMMULITE TNA Sensitivity Evaluation

Instrument: Immulite

Assay Type: **S** (Enter S for sandwich, C for competitive)

Replicates of the zero (CPS):

1	348405
2	355015
3	349779
4	361619
5	333370
6	340131
7	299164

8
9
10
11
12
13
14
15
16
17
18
19
20

AVG 341069
SD 20667
CV 6.1%

Four Parameters:

P1	38000000
P2	280000
P3	2920
P4	-1.04

Slope 0.9803041
Intercept -50903.32

Analytical Sensitivity

Sample	CPS	Dose
1SD	361736	2.438
2SD	382402	4.418
3SD	403069	6.364
4SD	423736	8.288

Date(s) Performed:
Performance Specification:

Laboratory Criteria:

Acceptable: **Y** N

Reviewed & Approved by:
Signature/Date:

Janani Prabakaran, Ph.D.
6-15-99

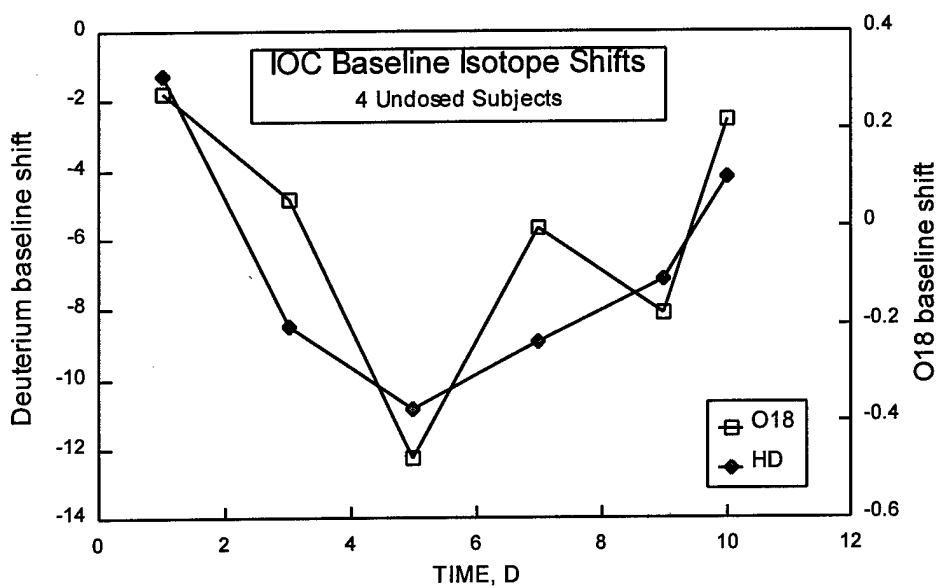
Pennington Biomedical
 Clinical Research Lab
 DPC Immulite
 Within Run Precision

TEST

TNF-A		
	Cyto 1	Cyto 2
	102.0	443.0
	94.1	461.0
	97.3	455.0
	96.9	446.0
	107.0	446.0
	103.0	452.0
	103.0	473.0
	95.2	482.0
	93.7	459.0
	102.0	448.0
Mean	99.42	456.5
SD	4.546989	12.67763
CV	4.573516	2.777136

Package	34	327
SD	1.2	8.6
CV	3.5	2.6
Sens	1.7	
Units	pg/ml	

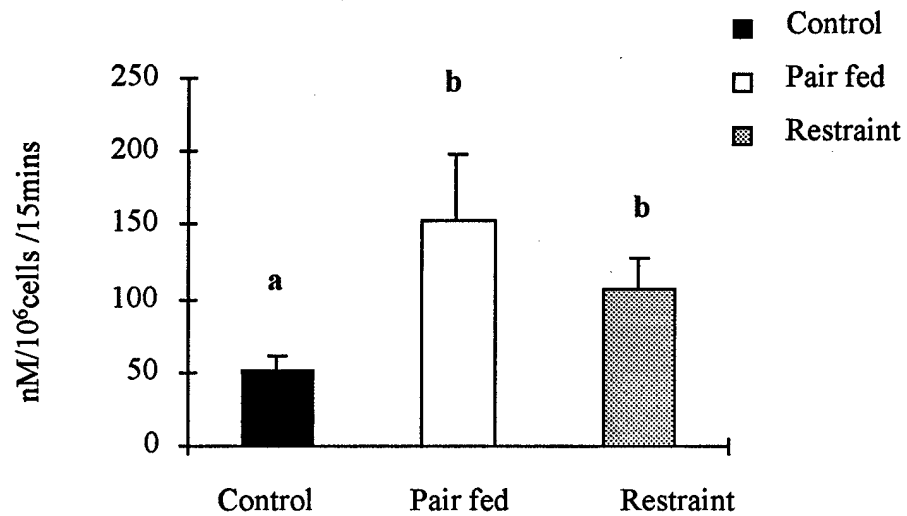
APPENDIX III
STABLE ISOTOPE LABORATORY



APPENDIX IV

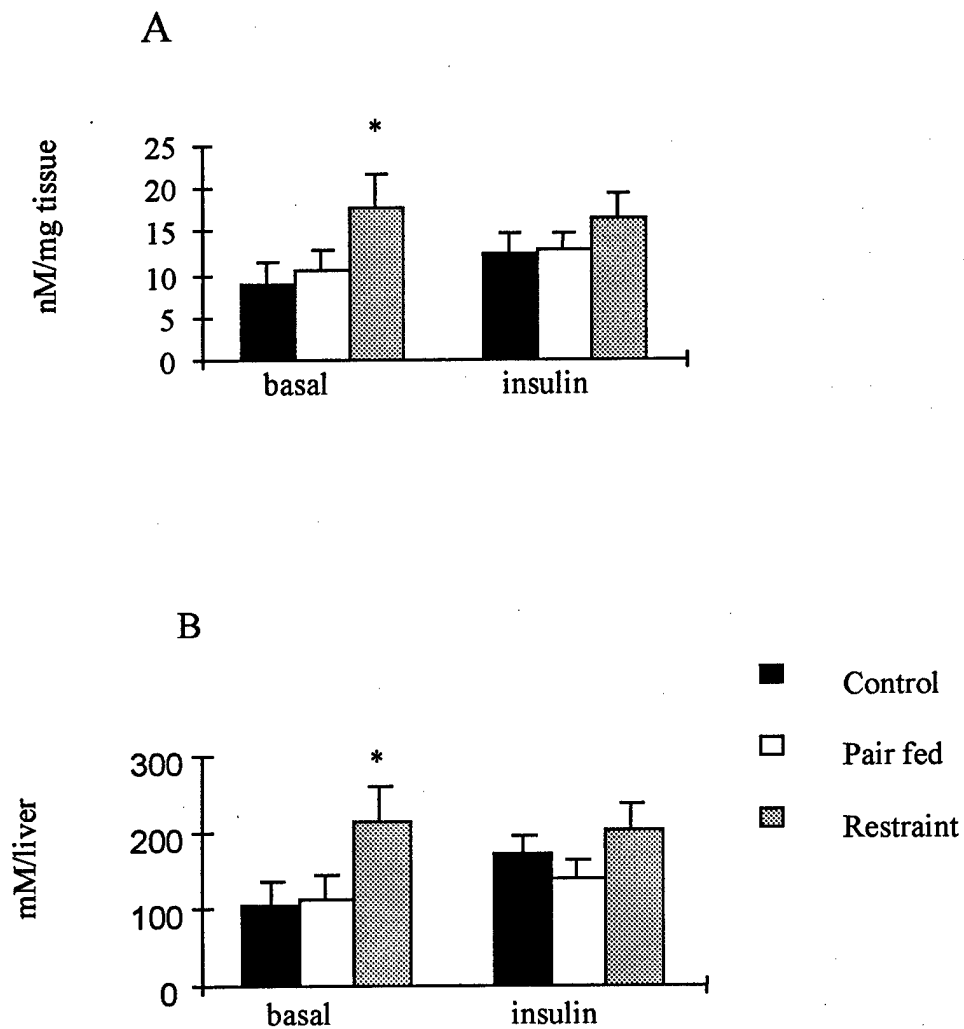
STRESS, NUTRITION AND MENTAL PERFORMANCE

Figure 1.



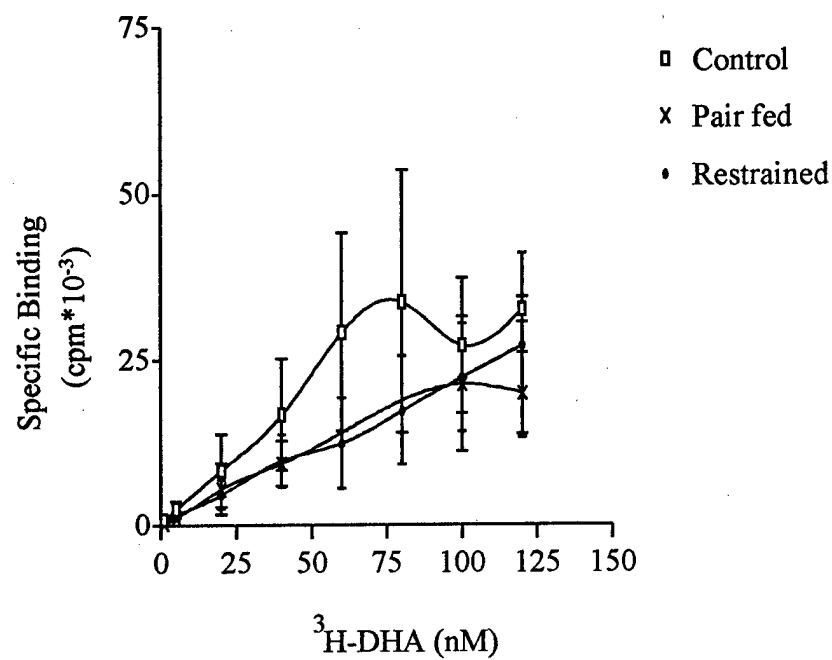
Glucose transport in isolated hepatocytes from control, pair fed and restraint rats. Data are means + SEM for groups of 10 rats. Pair fed and restraint rats had significantly higher rates of glucose transport than control rats. Values that do not share a common letter are significantly different ($P < 0.05$).

Figure 2.



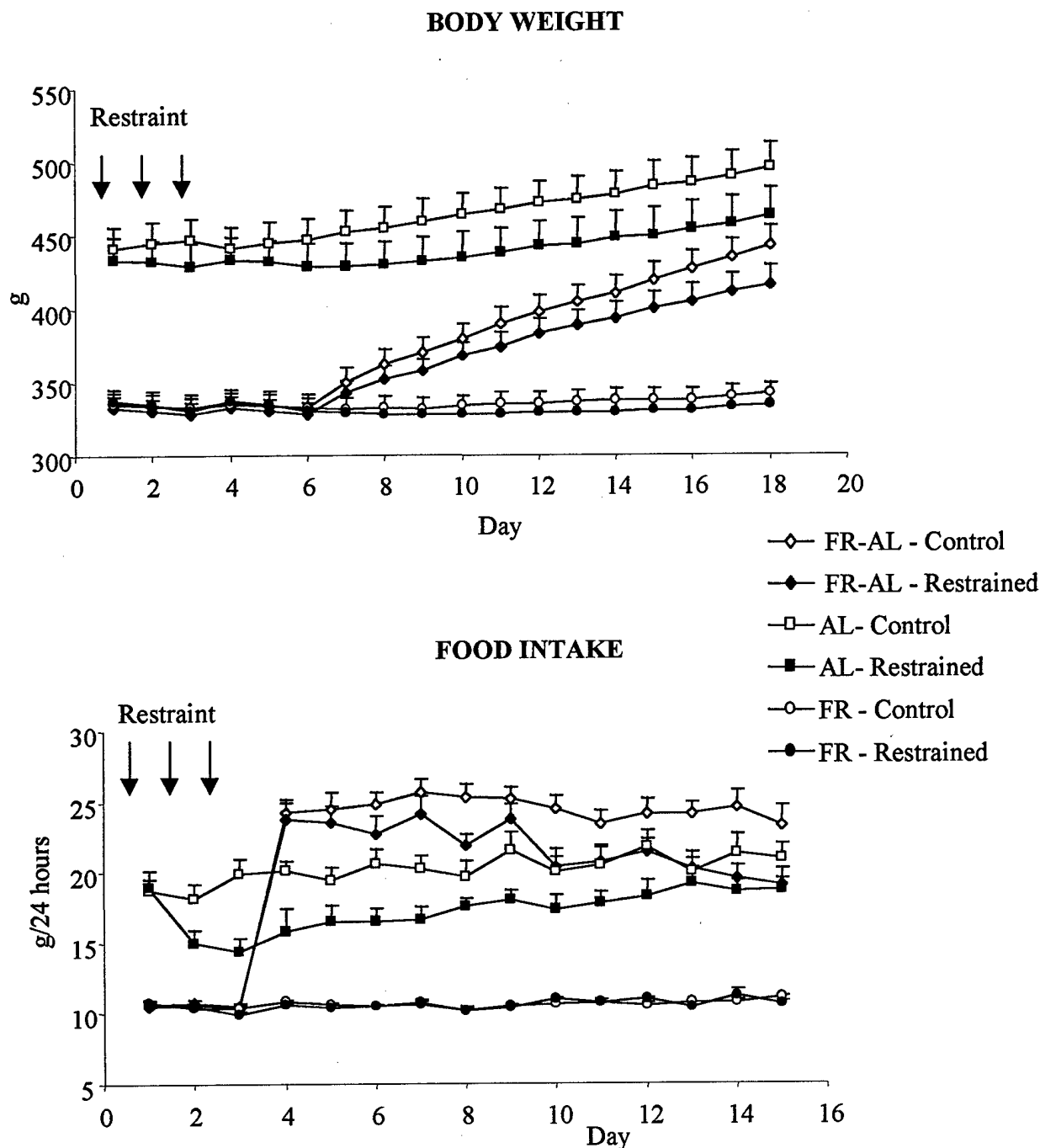
Glycogen synthesis in liver slices from control, pair fed and restraint rats. Data are means \pm SEM for groups of 8 rats. Panel A: glycogen synthesis calculated as nM glycogen /mg tissue. Panel B: glycogen synthesis calculated as mM glycogen/liver. Restraint rats had a significantly higher levels of glycogen synthesis than control or pair fed rats, as denoted by *

Figure 3.



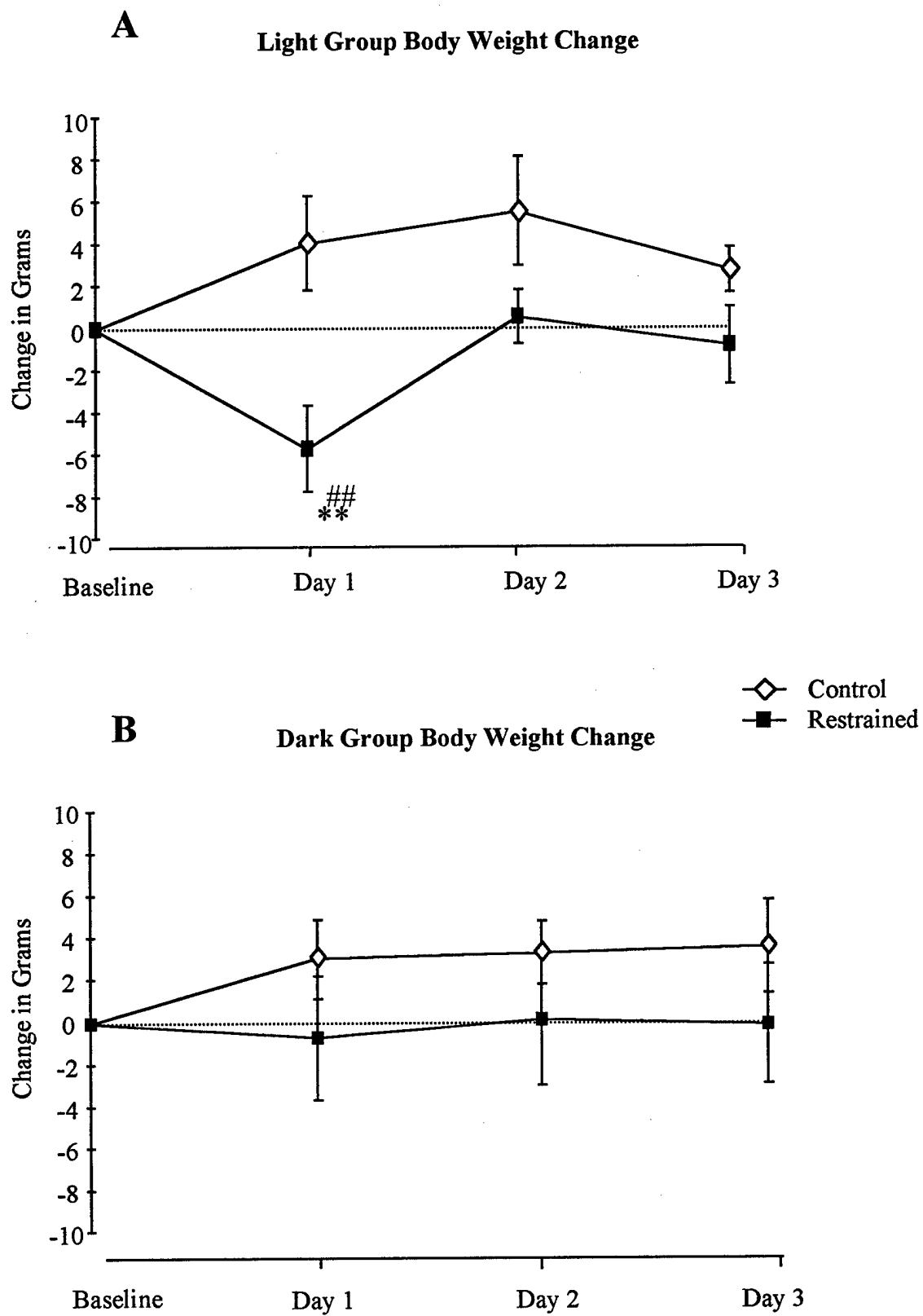
Specific binding of ^3H -DHA to adipose plasma membrane of control, pair fed and restraint rats. Data are mean \pm SE for 5 or 6 rats per group.

Figure 4



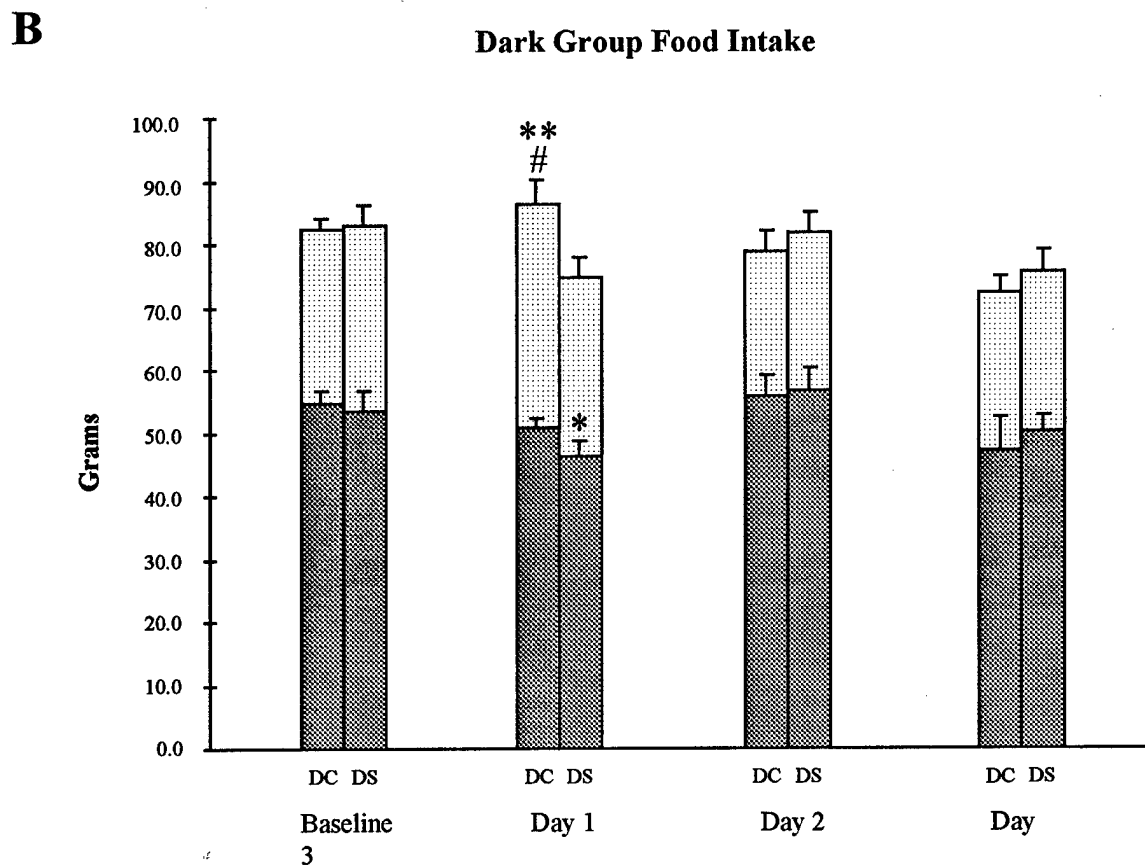
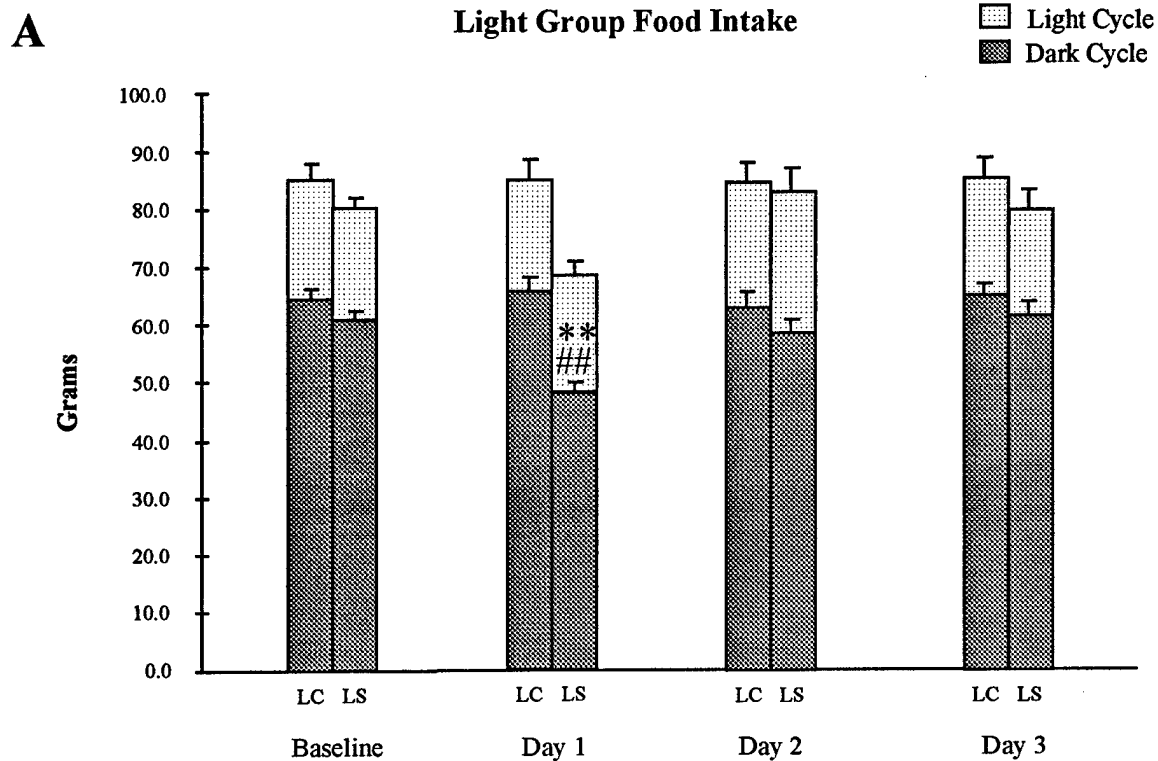
Daily food intakes and body weights of groups of 10 rats that were either restrained (R) or were non-restrained controls (C). Ad libitum fed rats (AL) were allowed free access to food throughout the study. FR rats were restricted to 50% of normal intake throughout the study. FR-AL rats were food restricted until the end of restraint and were then allowed free access to food.

Figure 5



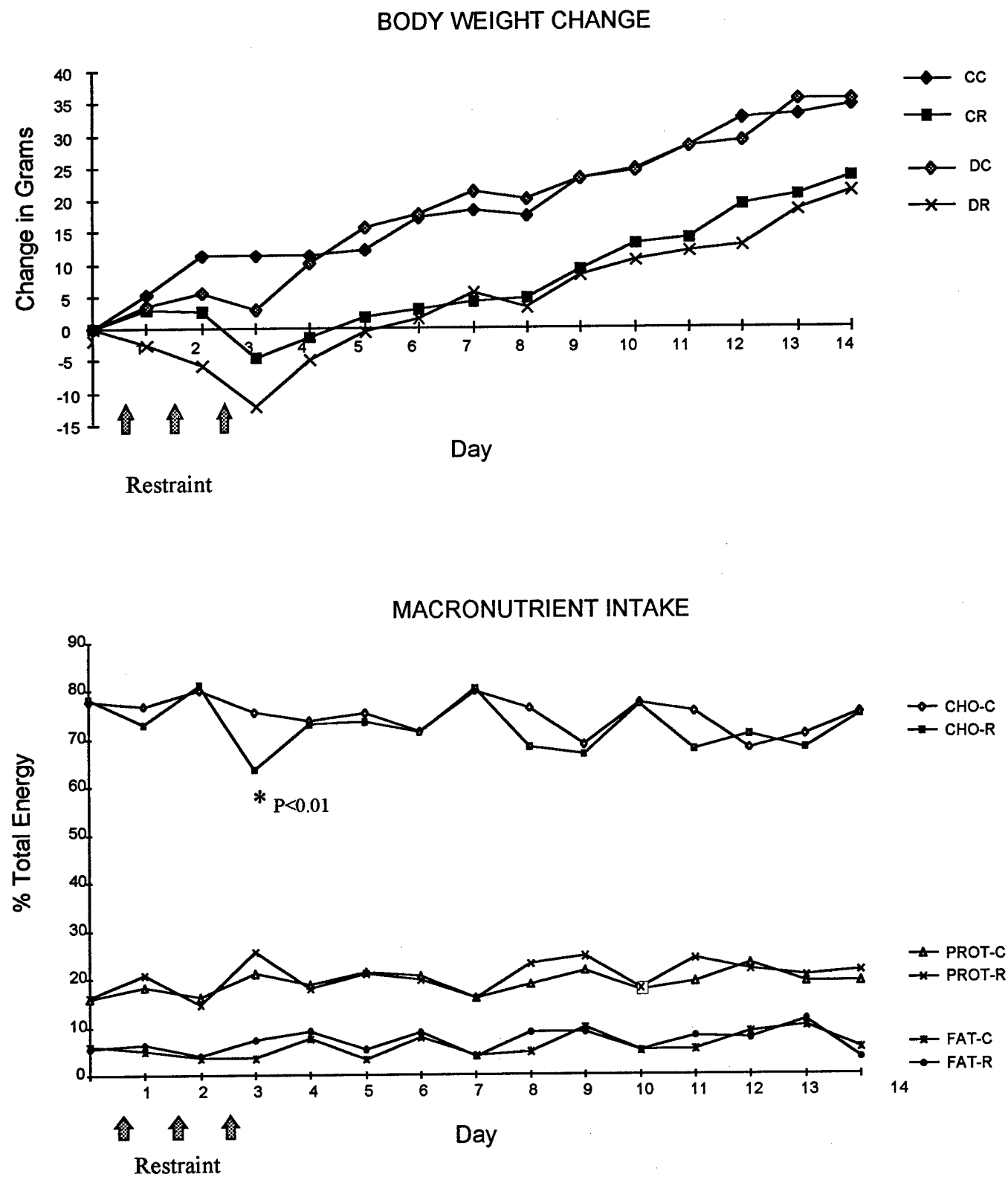
Data are means + sem for 8 rats. * indicates a significant difference from baseline and # indicates a significant difference from controls

Figure 6



Data are means + sem for 8 rats. * indicates a significant difference from baseline and # indicates a significant difference from controls

Figure 7



Data are means + sem for groups of 8 control rats (C) or rats restrained (R) for 3 hours on days 0-2.

Figure 8

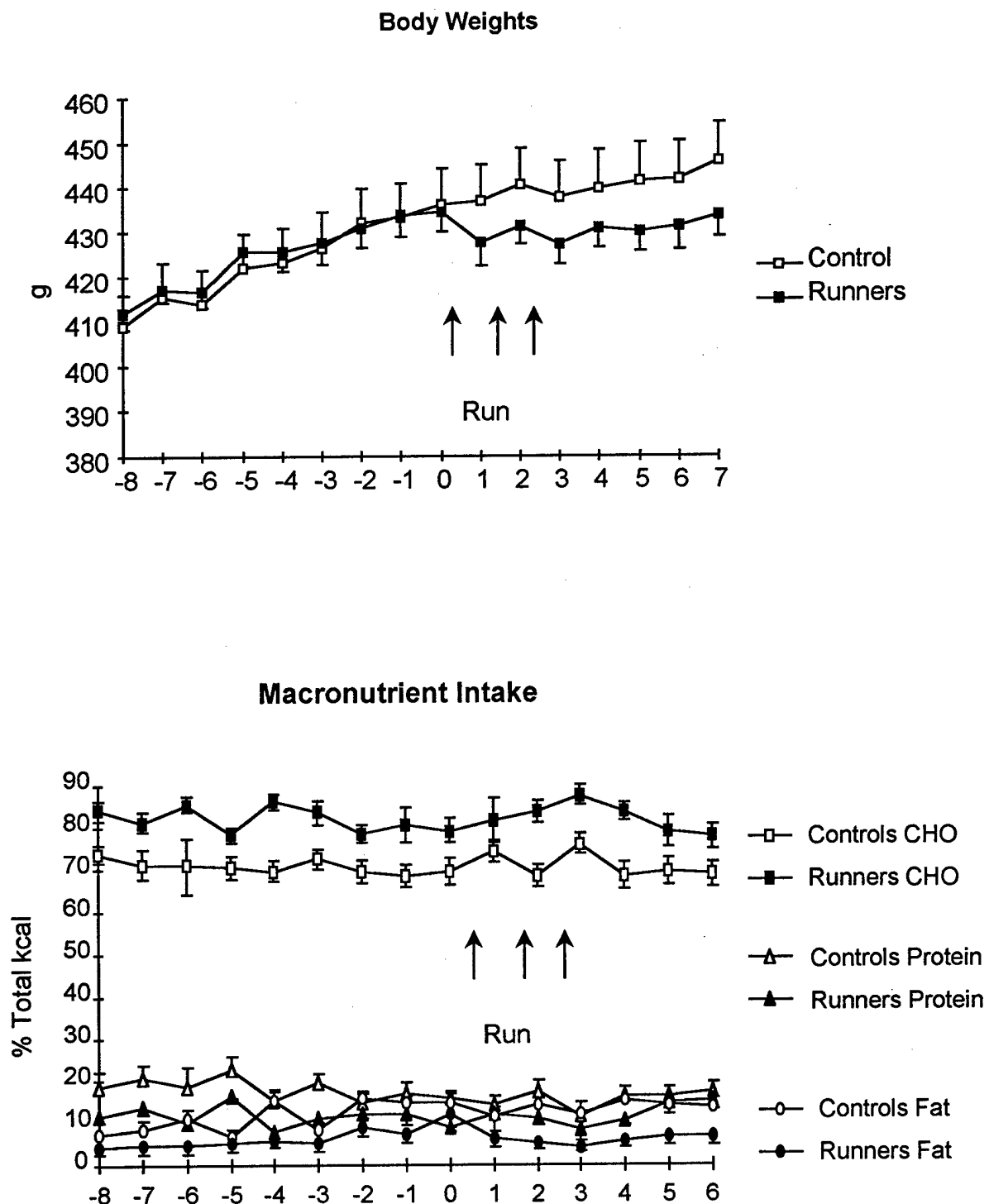
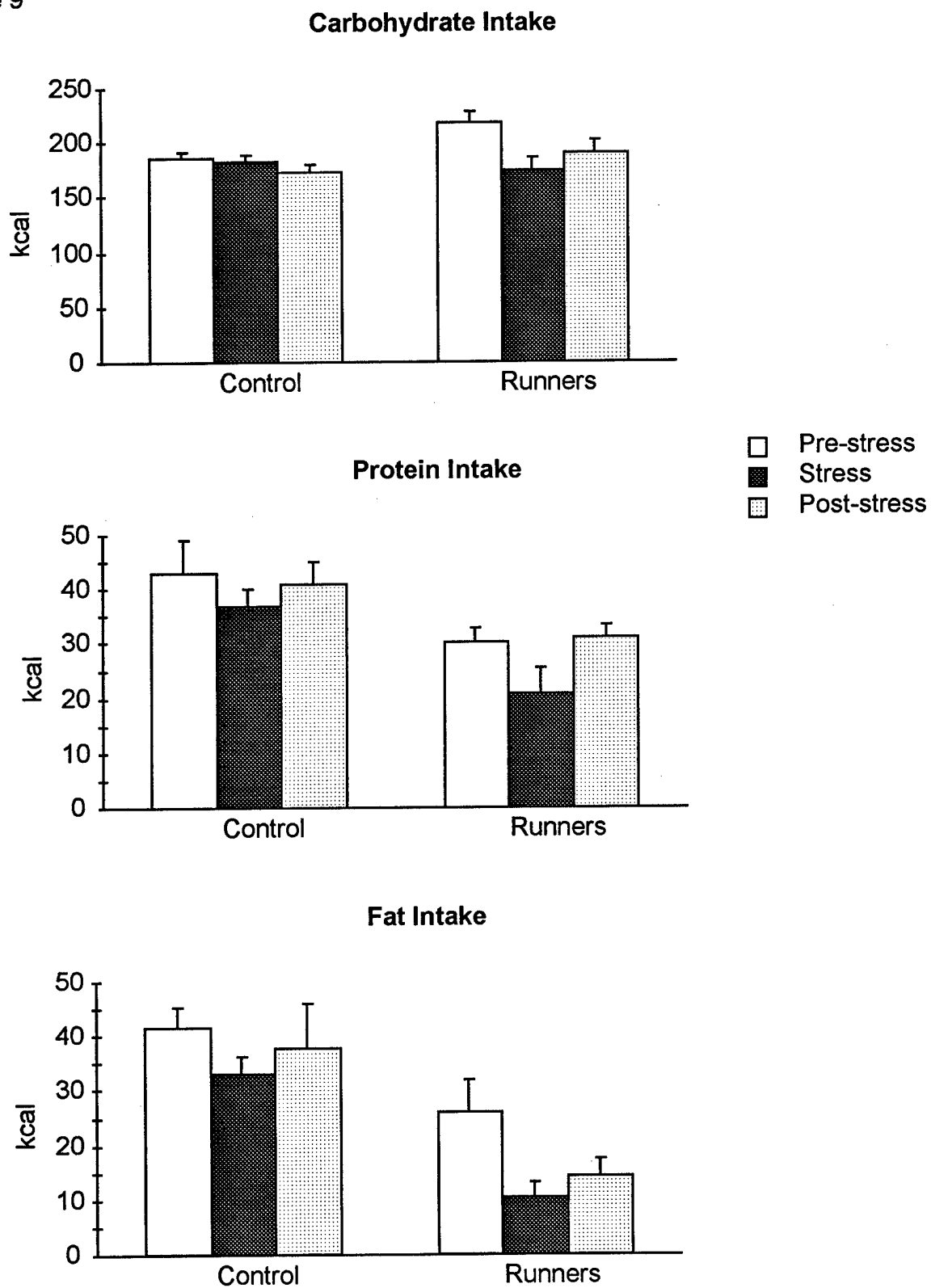


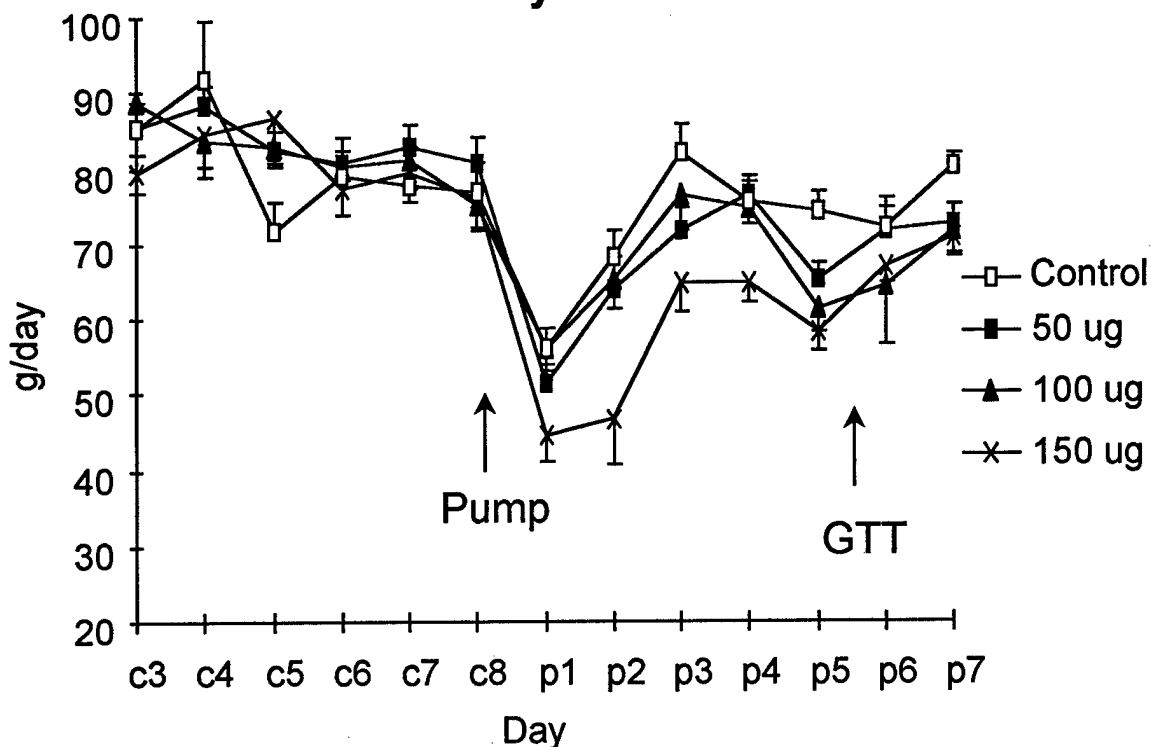
Figure 9



Data are means \pm sem for 7 rats. Intake is compared over 3 day periods during pre-stress, running and post-stress intervals.

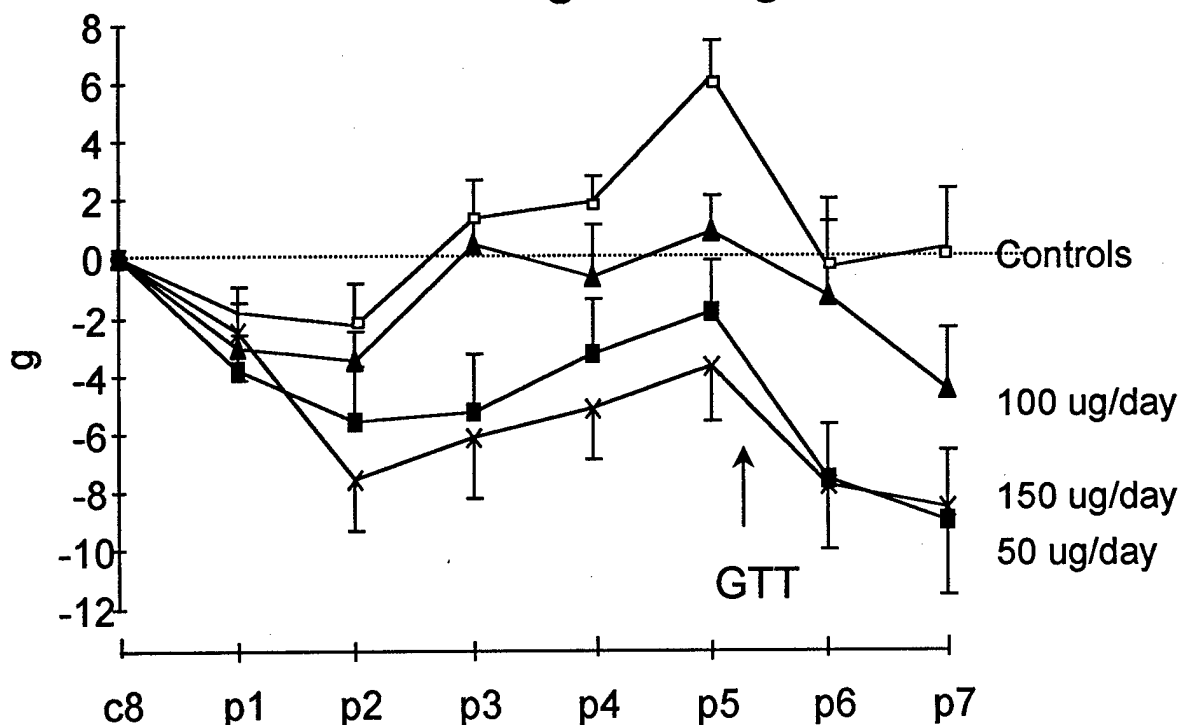
Figure 10

A: Daily Food Intake



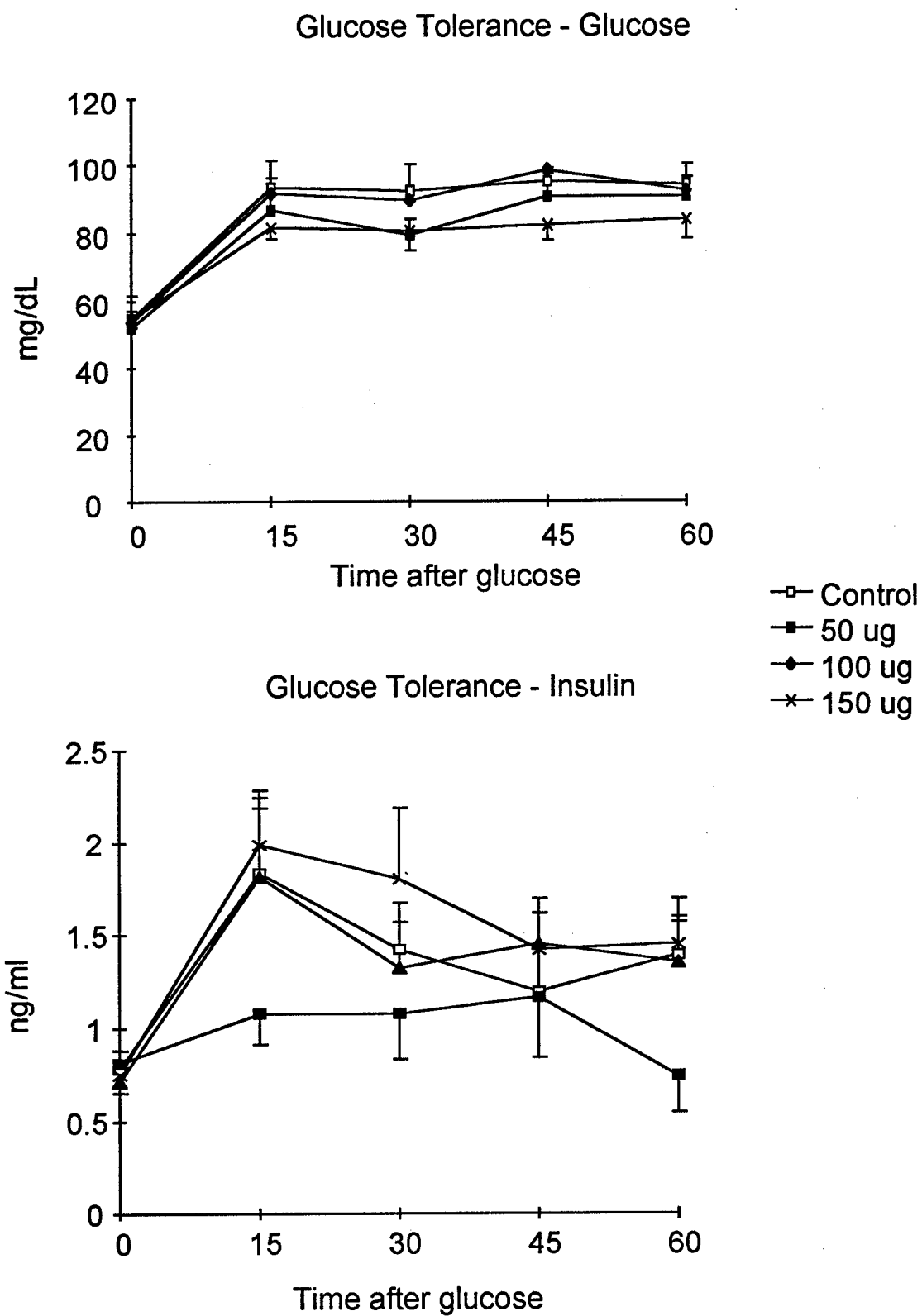
Daily food intake for groups of 8 rats fed a liquid diet and infused with leptin from an ip Alzet pump on days P1 to P7.

B: Weight Change



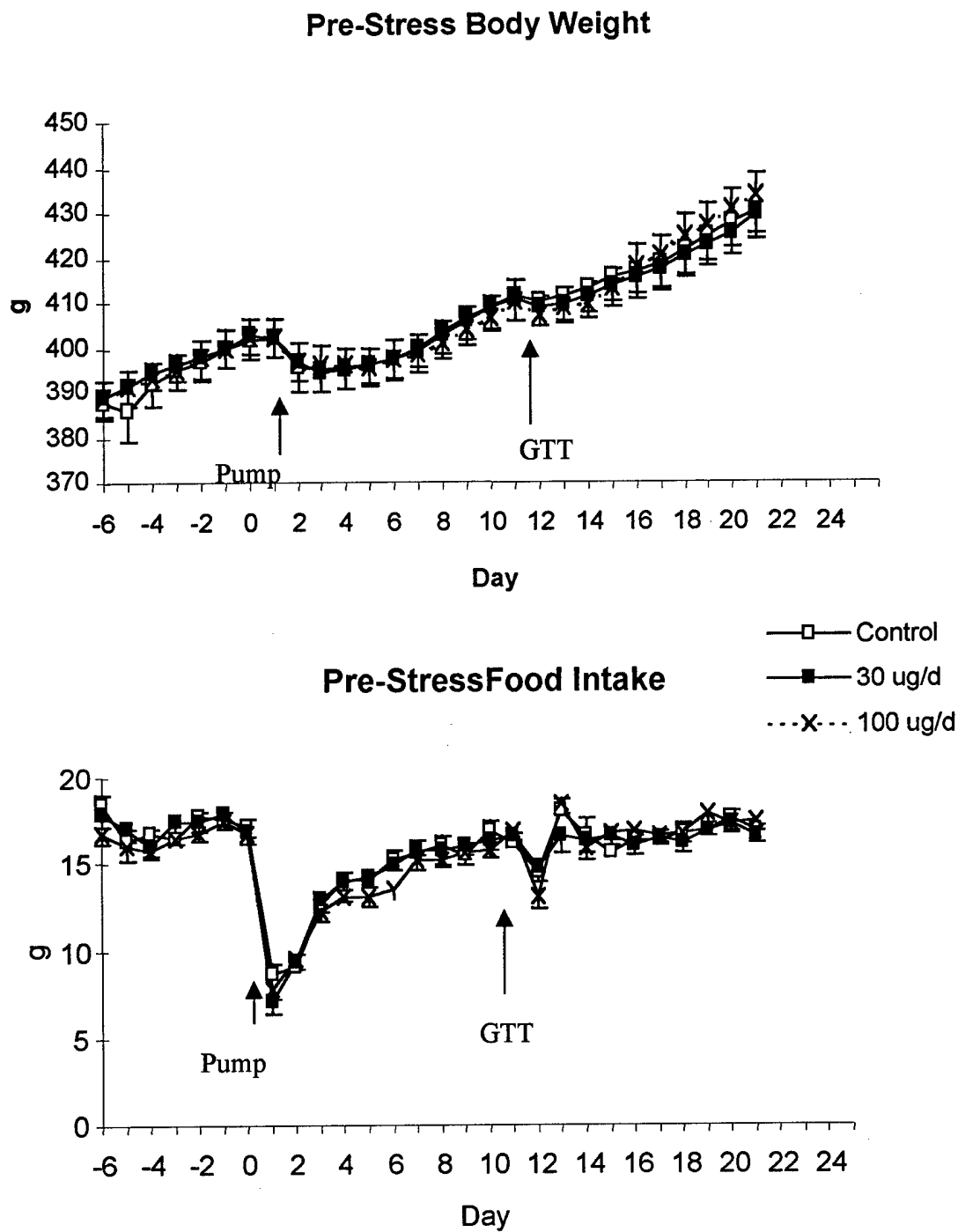
Weight change from baseline (C8) for groups of 8 rats fed a liquid diet and infused with leptin from an ip Alzet pump on days P1 to P7.

Figure 11



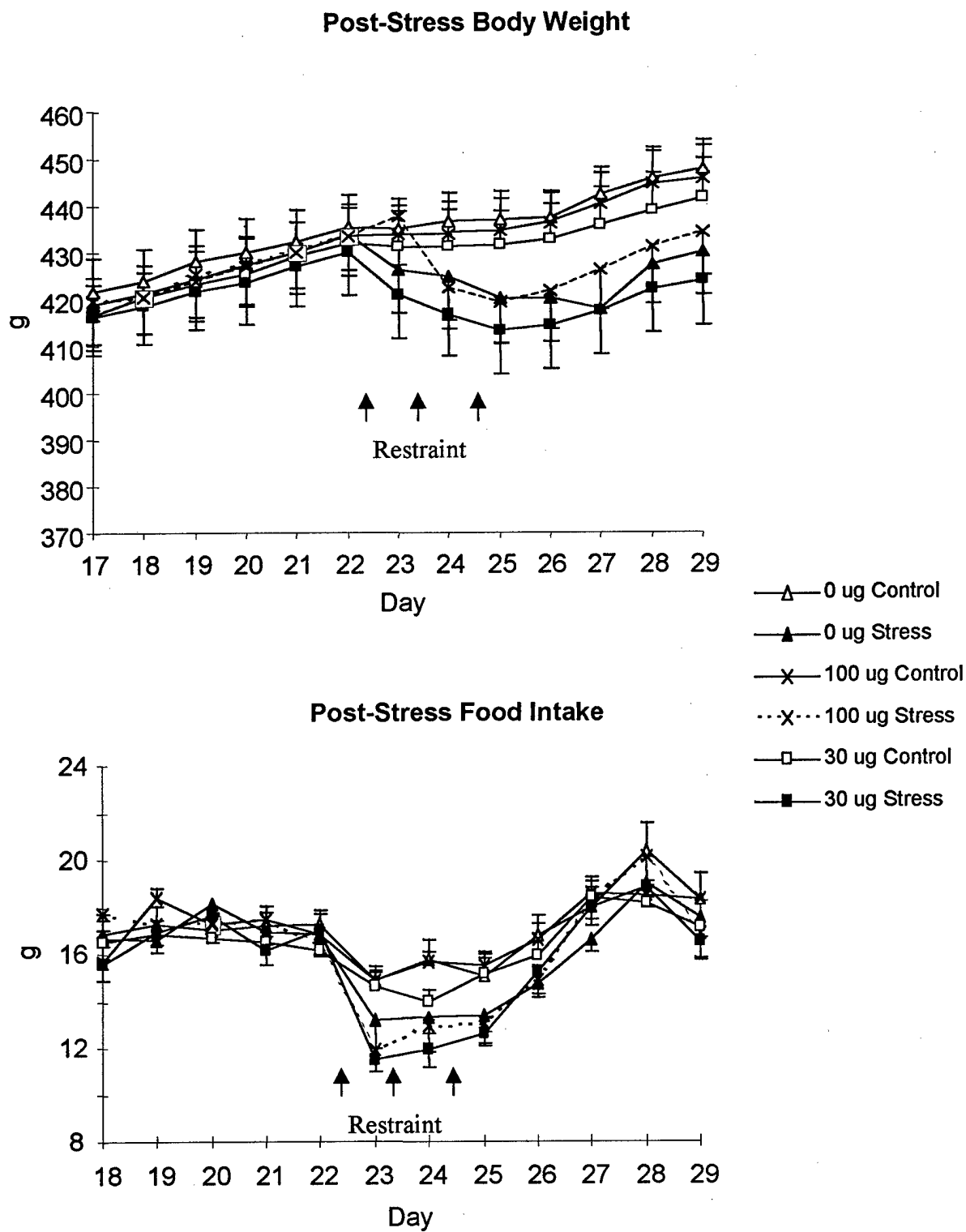
Serum glucose and insulin during an oral glucose tolerance test performed on day 5 of leptin infusion. Data are means + sem for 8 rats

Figure 12:



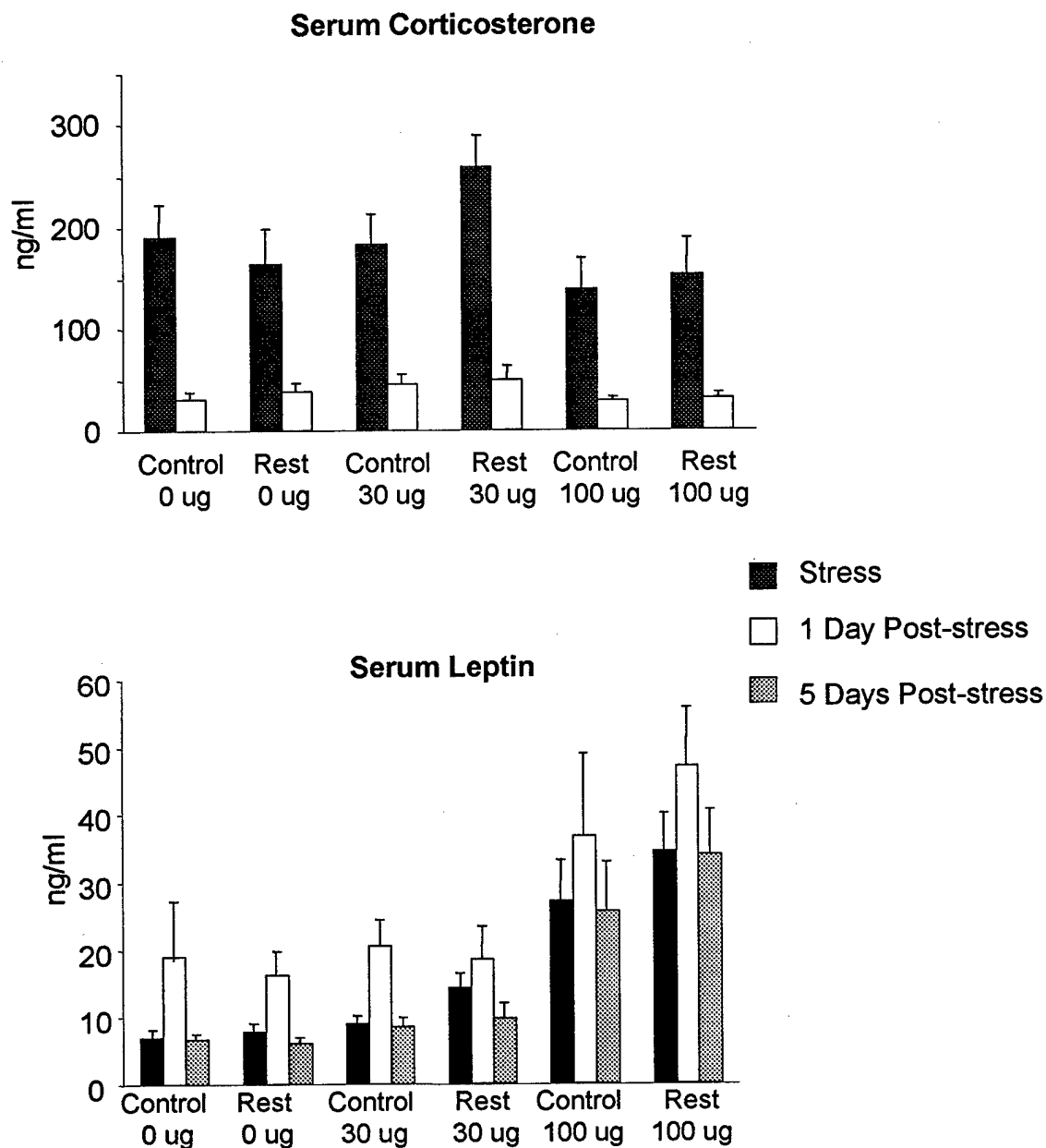
Data are means + sem for groups of 8 rats. There were no effects of leptin infusion on either food intakes or body weights of the rats.

Figure 13:



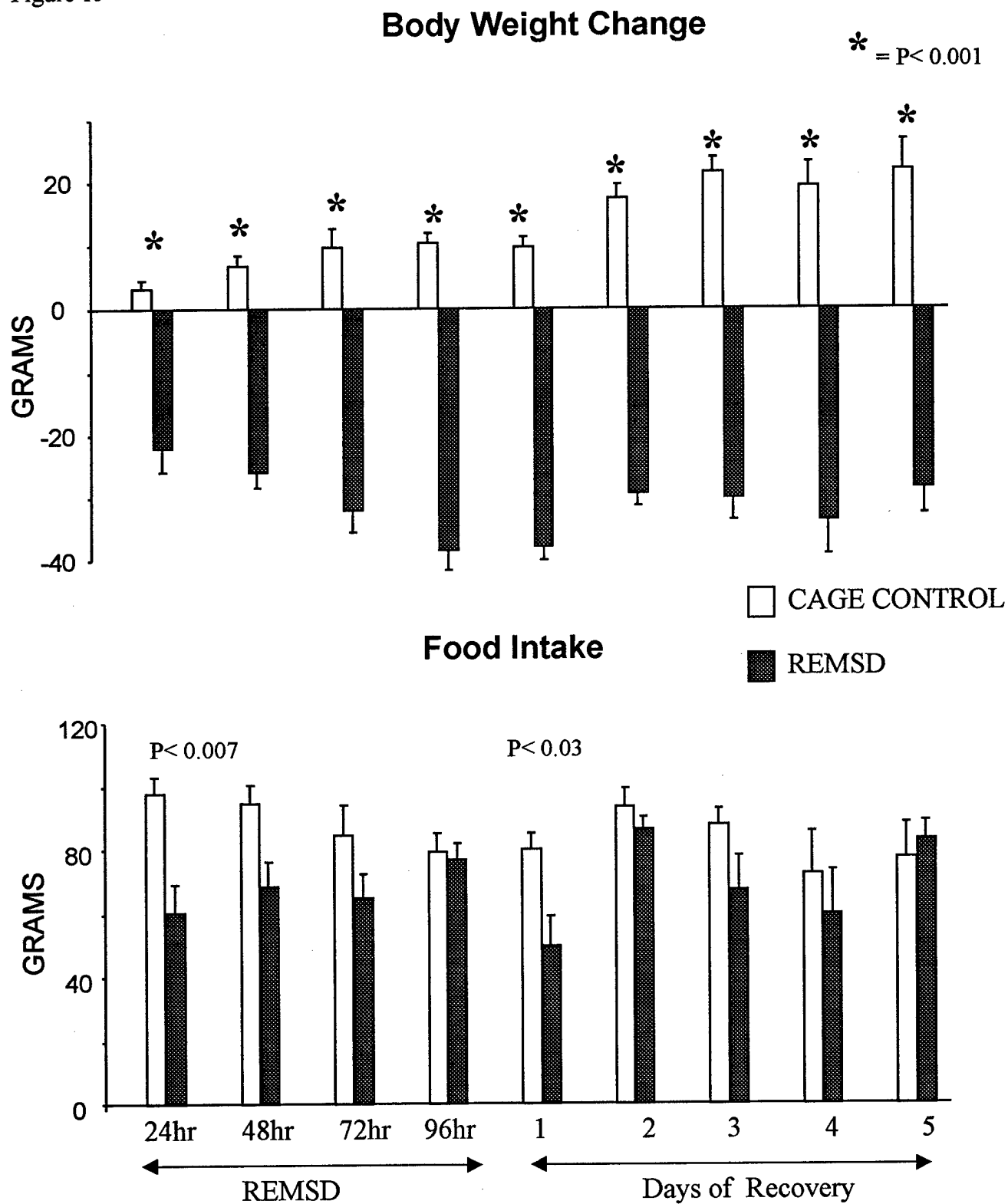
Data are means + sem for groups of 8 rats. Restraint caused significant reductions in food intake and body weight for all three treatment groups.

Figure 14:



Data are means + sem for groups of 8 rats. Blood was collected after 1 hour of restraint on the first day of repeated restraint for the stress sample and at the same time on the day after stress. The 5 days after stress sample is truck blood collected when the rats were decapitated.

Figure 15



Data are means + sem for groups of 8 rats. Data was analyzed by a two-way ANOVA followed by the Duncan's Multiple Range test. Significance was set at $P < 0.05$.

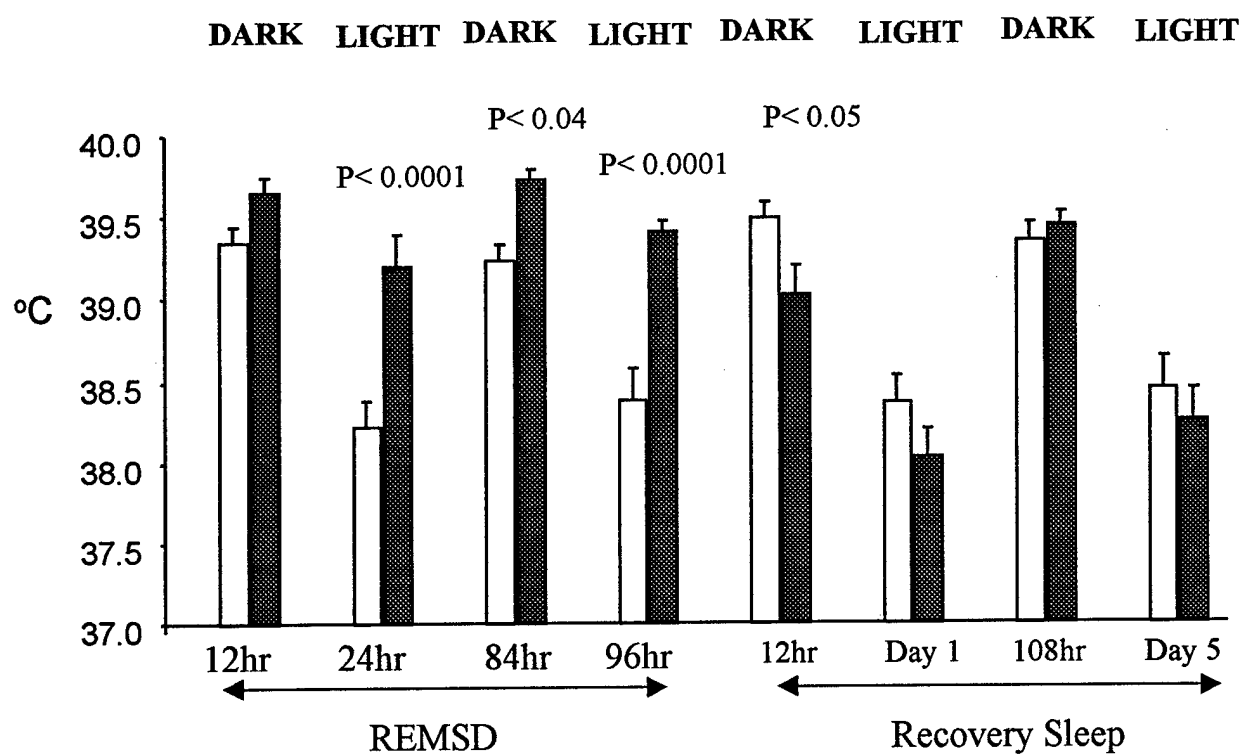
Figure 16

Treatment $P < 0.001$
 Time $P < 0.001$
 Interaction $P < 0.001$

□ CAGE CONTROL

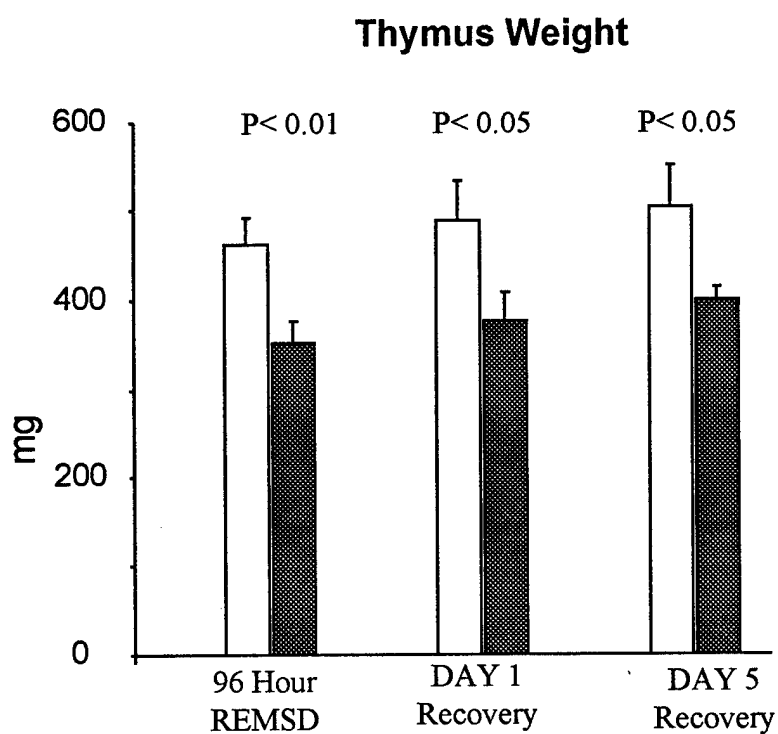
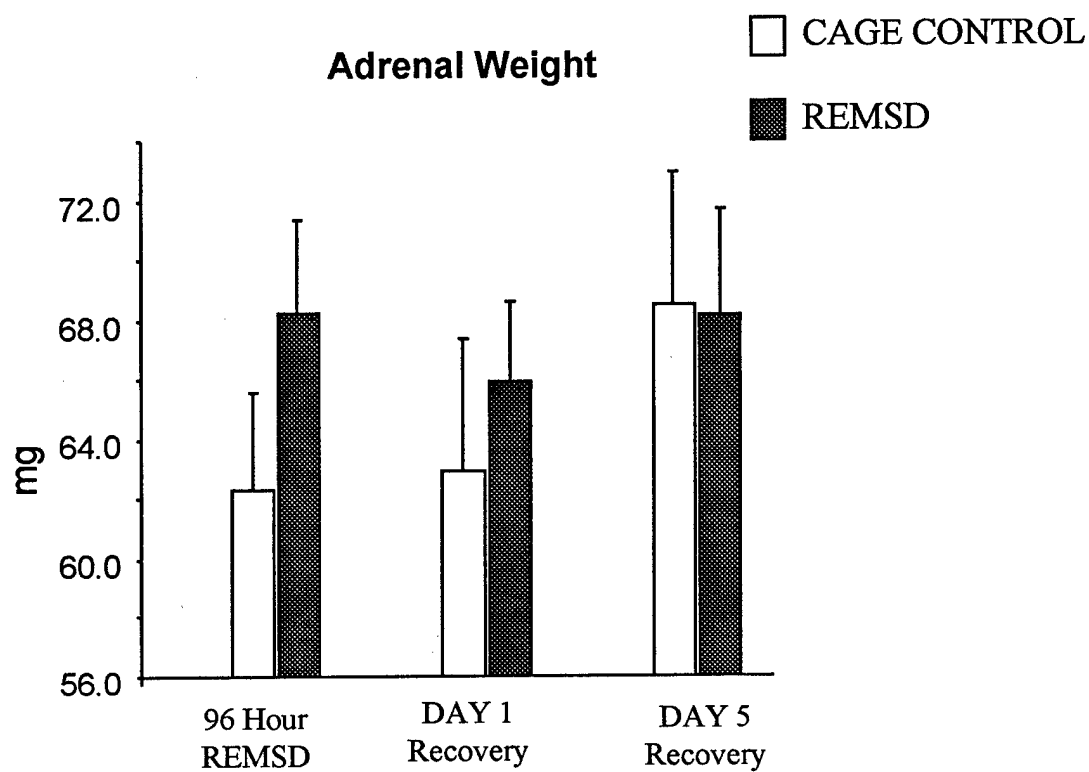
■ REMSD

Rectal Temperature



Data are means + sem for groups of 8 rats. Data was analyzed by a two-way ANOVA followed by the Duncan's Multiple Range test. Significance was set at $P < 0.05$.

Figure 17



Data are means + sem for groups of 8 rats. Data was analyzed by a two-way ANOVA followed by the Duncan's Multiple Range test. Significance was set at $P < 0.05$.

Figure 18

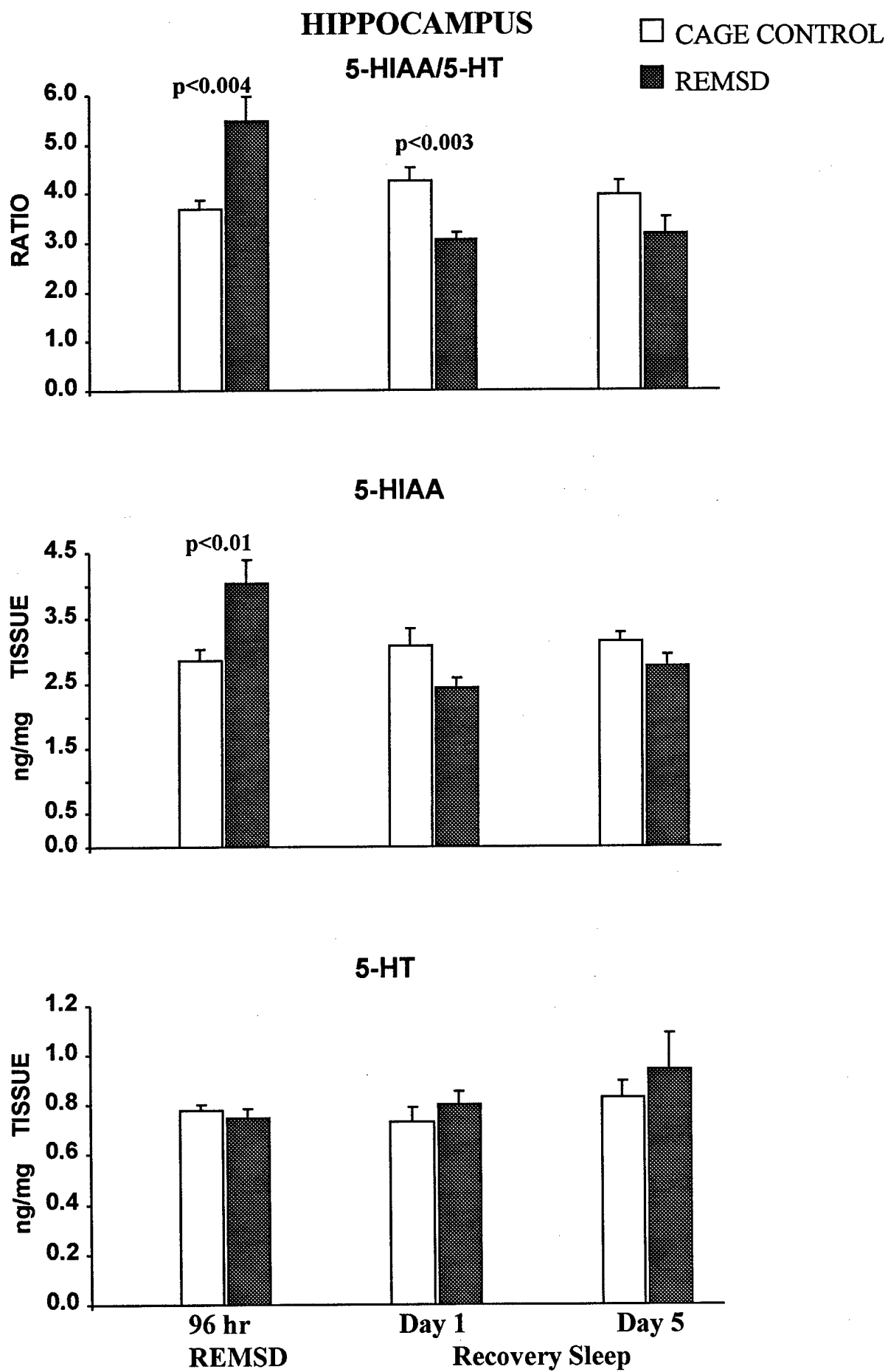
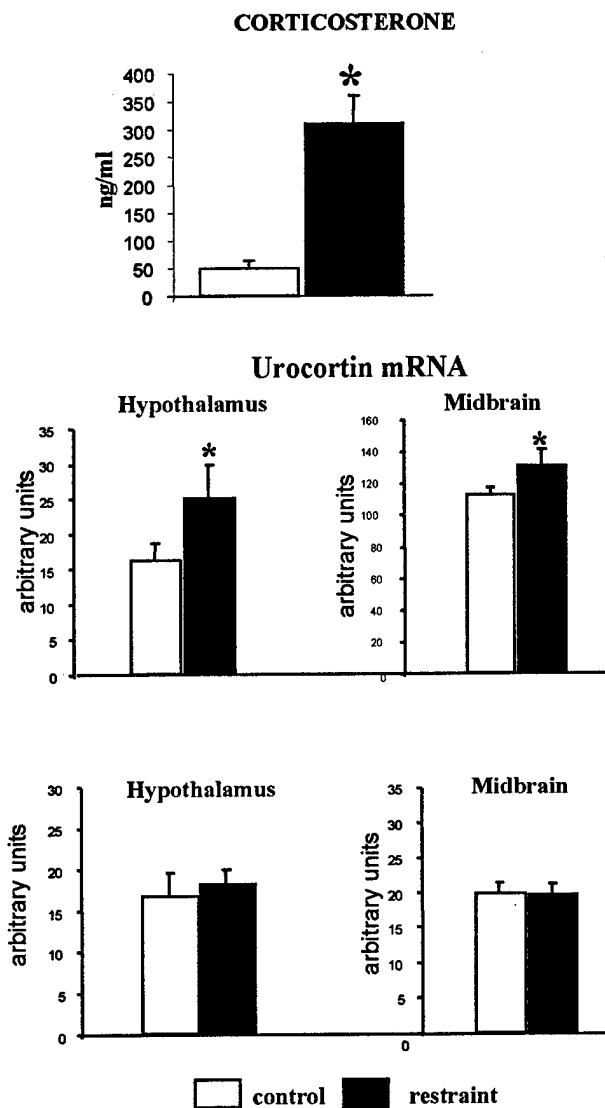
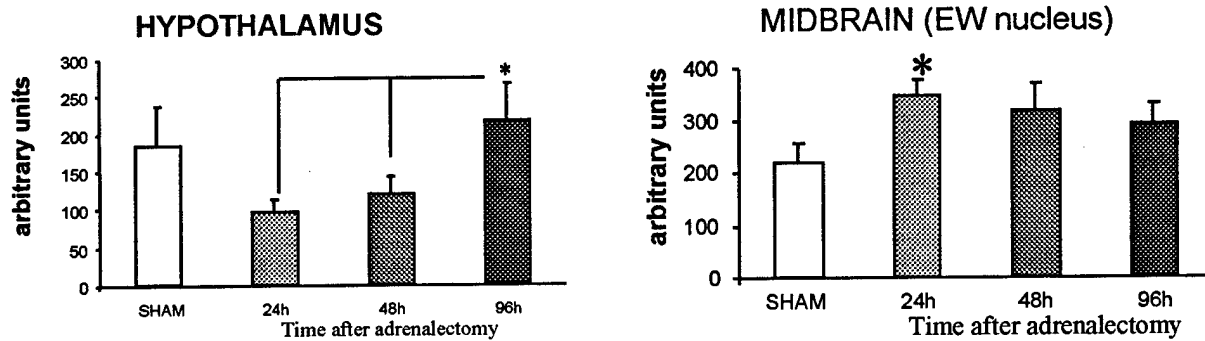


Figure 19



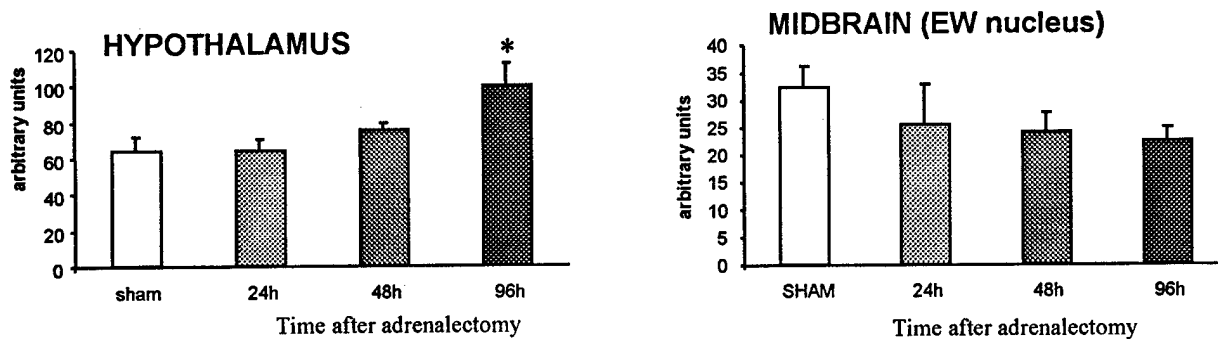
The effect of 1 hour restraint on serum corticosterone (upper panel), hypothalamic and midbrain UCN mRNA (middle panel) and CRH mRNA (lower panel) levels in rats. Values are standardized to arbitrary units. Brain regions were processed separately. *-significantly different from control animals, $P < 0.05$, $n = 8$.

Figure 20



The effect of ADX on UCN mRNA levels in the hypothalamus and midbrain. Values are standardized to arbitrary units. Brain regions were processed separately.

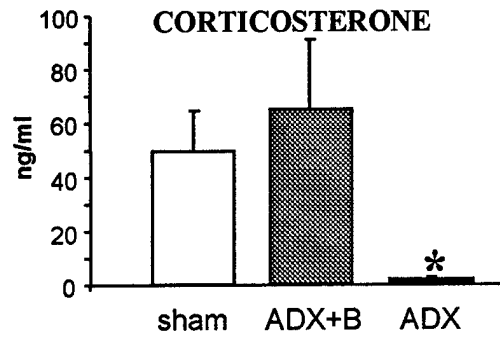
*-P<0.05, n=8.



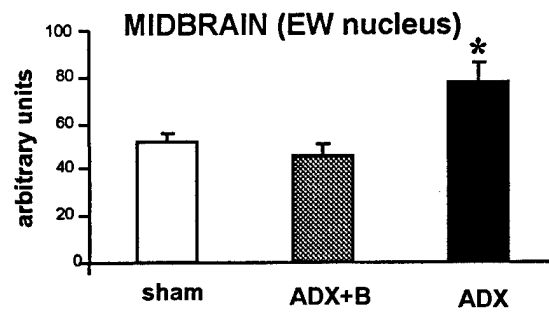
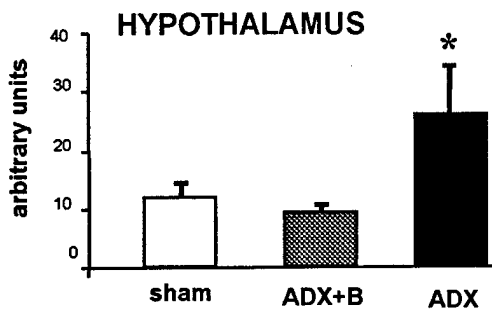
The effect of ADX on CRH mRNA levels in the hypothalamus and midbrain of rats. Values are standardized to arbitrary units. Brain regions were processed separately.

*-P<0.05, n=8.

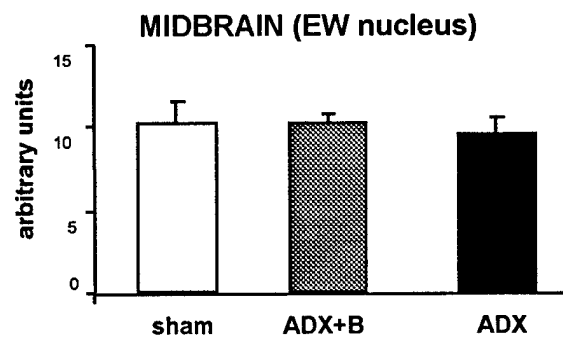
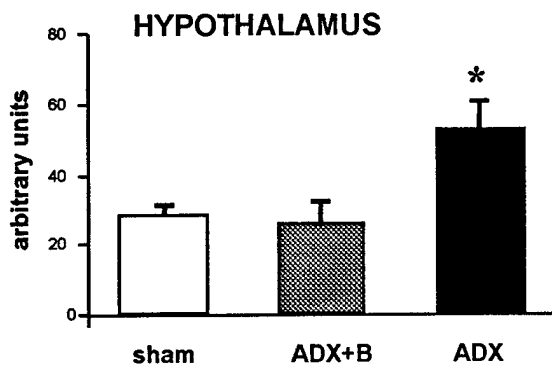
Figure 21



UCN

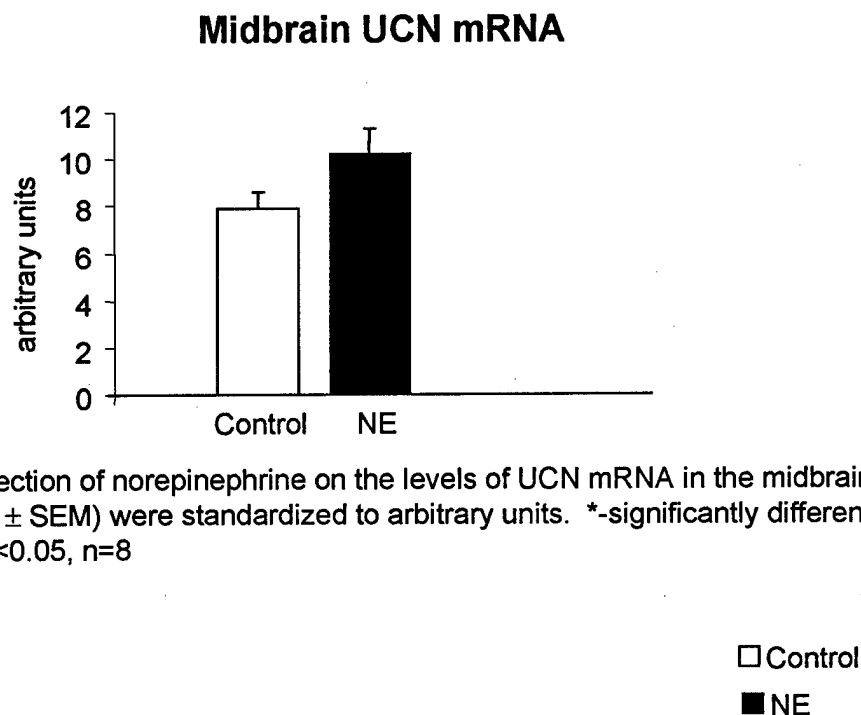


CRF

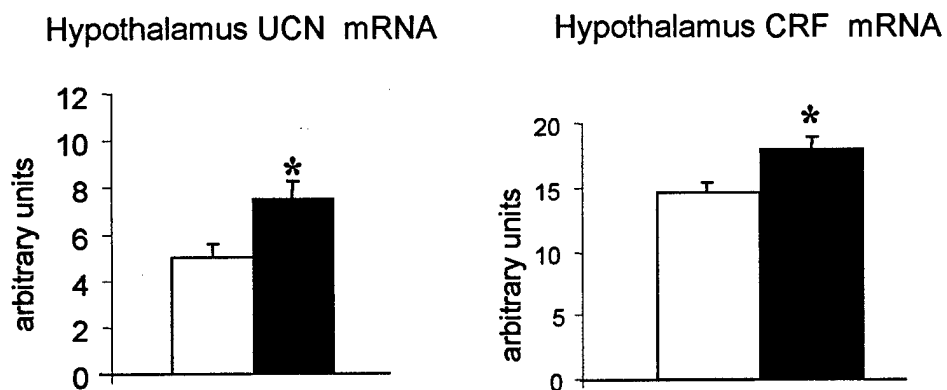


The effect of ADX and corticosterone replacement (ADX+B) on serum corticosterone and UCN and CRF mRNA levels in the hypothalamus and midbrain of rats. Values are standardized to arbitrary units. Brain regions were processed separately. *-significantly different from sham-operated animals, $P < 0.05$, $n = 8$.

Figure 22

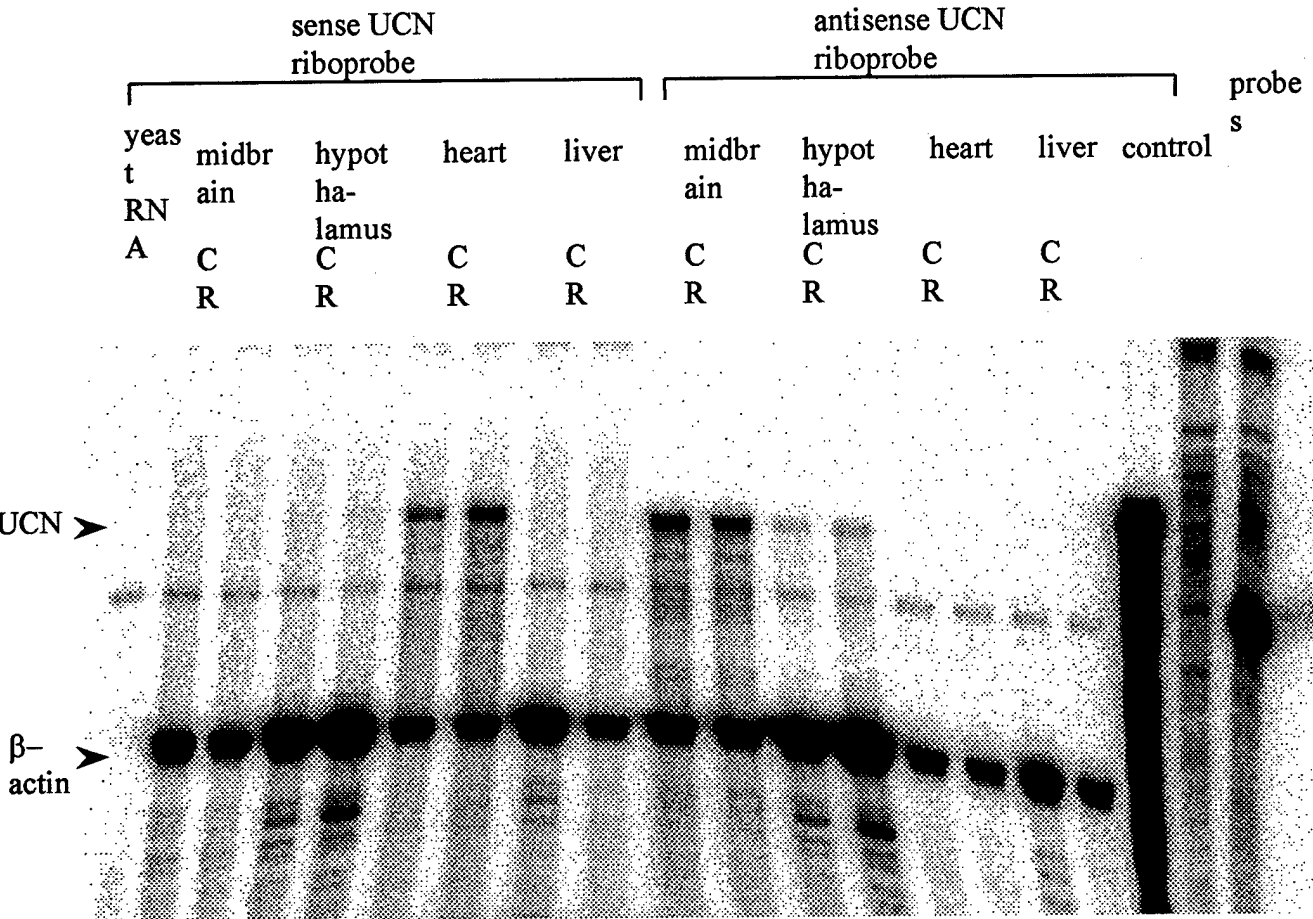


The effect of ICV injection of norepinephrine on the levels of UCN mRNA in the midbrain of rats. Values (means \pm SEM) were standardized to arbitrary units. *-significantly different from the control group, $P < 0.05$, $n = 8$



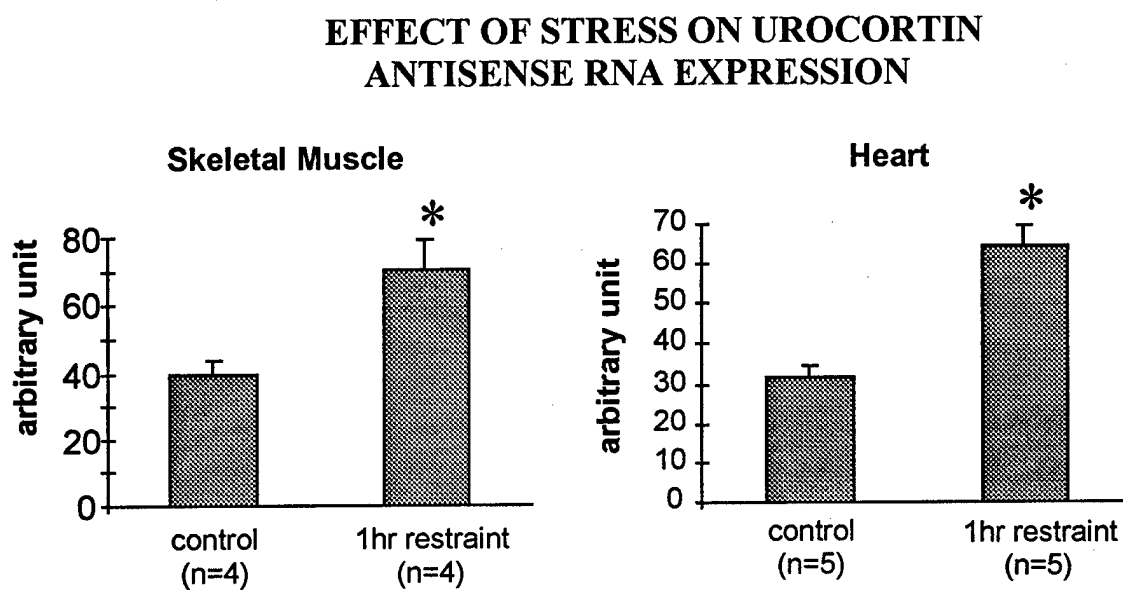
The effect of ICV injection of norepinephrine on the levels of CRF and UCN mRNA in the hypothalamus of rats. Values (means \pm SEM) were standardized to arbitrary units. *-significantly different from the control group, $P < 0.05$, $n = 8$

Figure 23



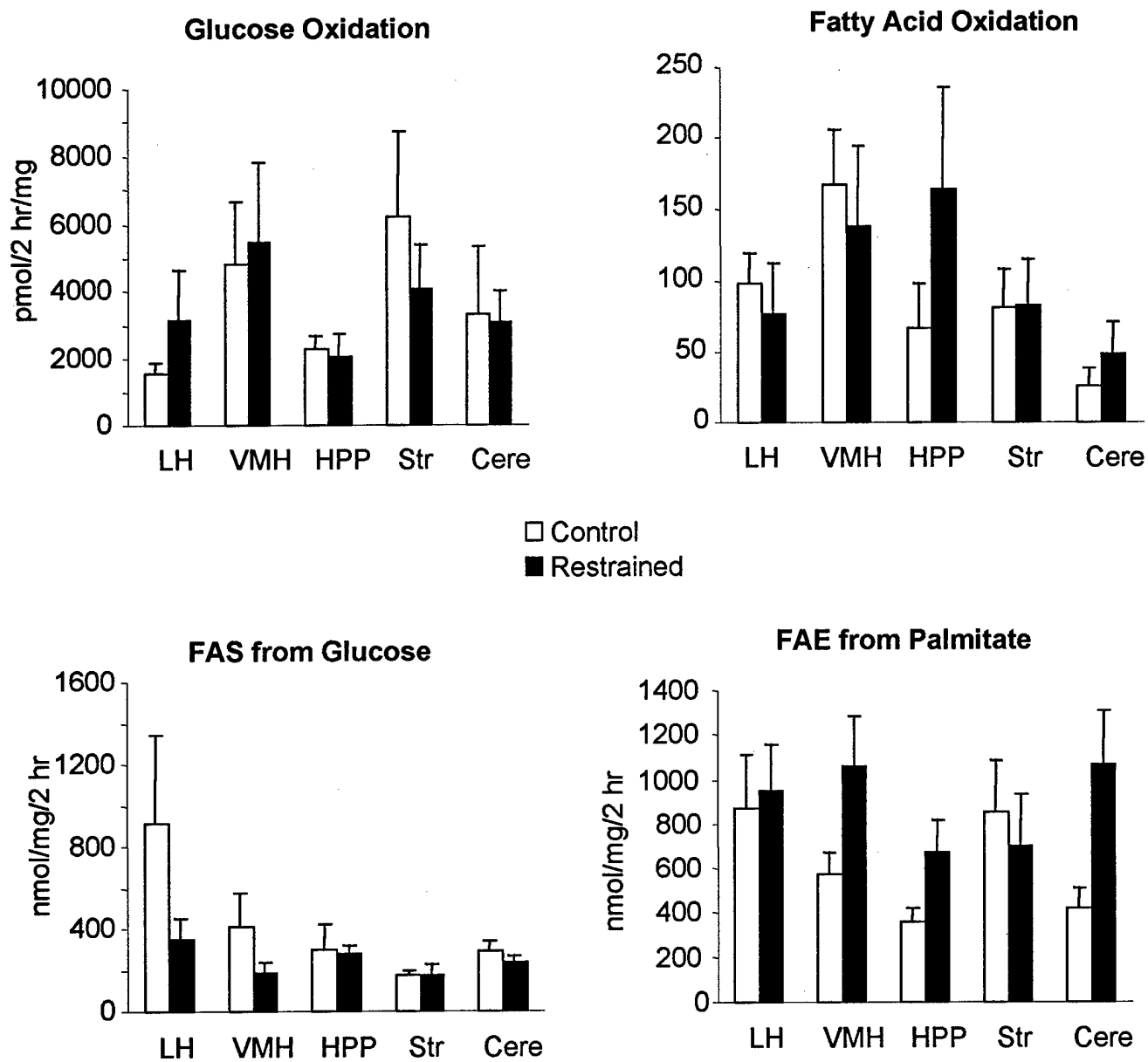
RNase analysis of RNA expression in tissue from control (C) rats and rats exposed to 1 hour restraint stress (R). The sense UCN riboprobe detected antisense message in heart and hypothalamus. The antisense probe detected sense message in midbrain and hypothalamus.

Figure 24



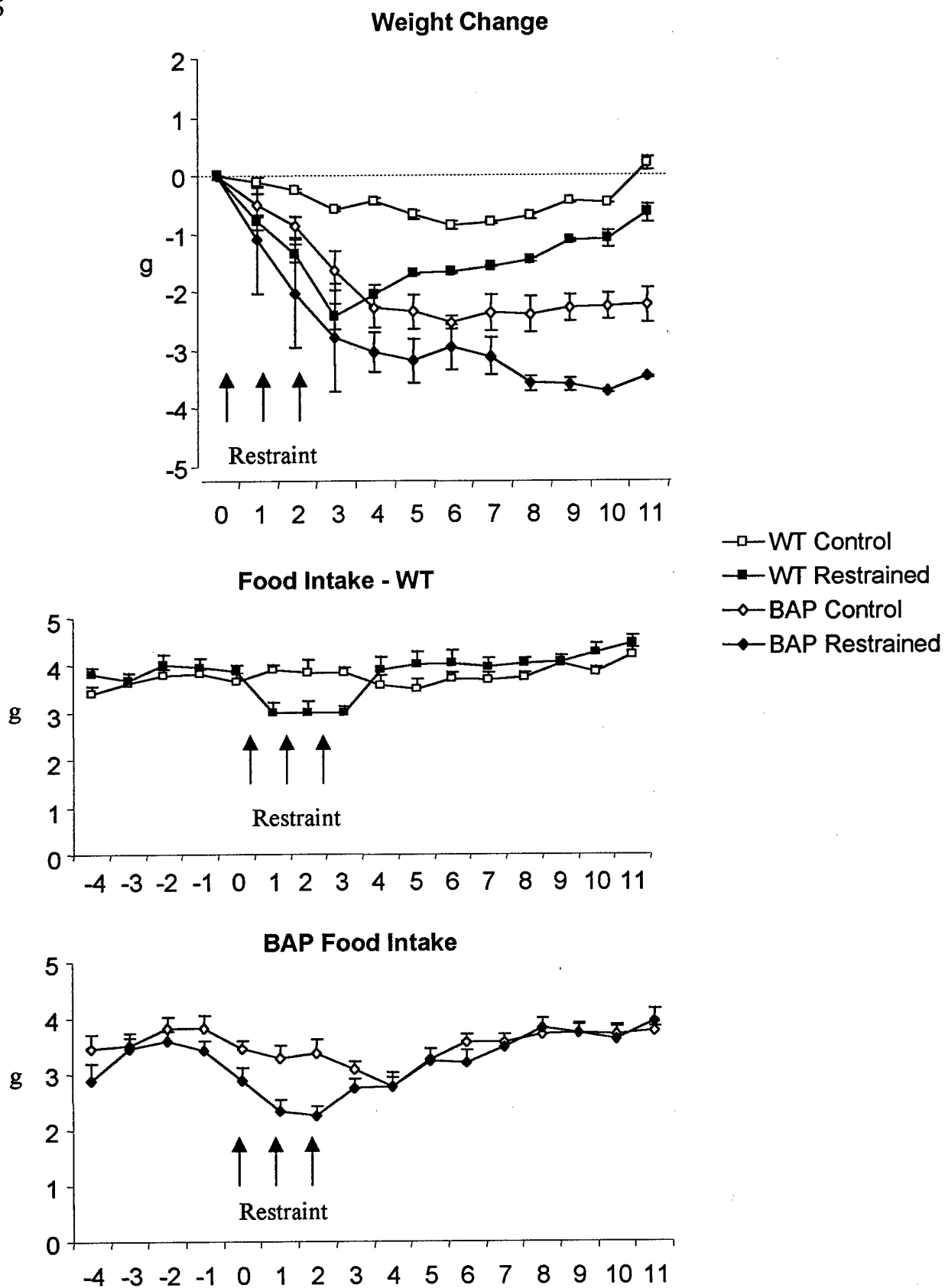
Data are means for 5 rats. Tissue was collected immediately after 1 hour of restraint stress. An asterisk indicates a significant difference in level of expression between control and stressed rats.

Figure 25



Data are means for 5 or 6 rats killed immediately after 3 hours of restraint stress

Figure 26



Data are means \pm sem for groups of 10 mice. The mice were restrained for 2 hours/day on days 0,1 and 2.

APPENDIX V

STRESS, NUTRITION AND WORK PERFORMANCE

NONE

APPENDIX VI

NUTRIENT DATABASE INTEGRATION LABORATORY

TITLE PAGE

Title: Incorporating new recipes into the Armed Forces Recipe File: Determination of acceptability by more cost effective means.

Authors:

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Alice E. Hunt, PhD, RD; Associate Professor of Nutrition and Dietetics, School of Human Ecology; Louisiana Tech University, Ruston, LA Zip

Alana D. Cline, PhD, RD; Assistant Professor for Research; Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124

Kelly Patrick, Culinary Research Associate; Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124

Donna H. Ryan, MD; Associate Executive Director for Science; Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808-4124

ABSTRACT

As part of a menu modification project to lower fat, cholesterol and sodium in soldiers' diets, new ethnic and breakfast items were developed and standardized for 100 portions. Acceptability data were collected after initial recipe development, during recipe validation at a collaborating university, and in an actual Army garrison. Acceptability was determined using a 9-point hedonic scale and products rating 6.0 or better in initial tests were prepared in an actual garrison setting. Acceptability data were compared among the test settings, ethnic categories, and food type. When grouped by ethnic categories, the acceptability ratings were more variable than when grouped by food type. We found ratings varied most between the development and validation settings (7.2 vs 6.6, $P < 0.05$), and least between the validation and actual Army setting (6.6 vs 6.6, ns). Since acceptability ratings were so similar between validation and Army garrison, we anticipate that future recipe development can continue without additional testing at an actual Army garrison allowing for considerable cost savings and more timely additions to the Armed Forces Recipe File.

INTRODUCTION

Since 1985, nutrition initiatives have been introduced into the Armed Forces Recipe Service, the Army Master Menu and the Army Food Service Program to provide soldiers with diets lower in fat, cholesterol and sodium. The Military Nutrition Division of the United States Army Research Institute of Environmental Medicine (USARIEM) conducted several garrison dining facility studies to assess soldiers' nutrient intakes (Carlson et al., 1987; Szeto et al., 1987; Szeto et al., 1989). It was apparent from these studies that in order to achieve Army Nutrition Initiative goals of reducing fat to 30% of energy intake and cholesterol to 300 mg/day, extensive revision of Armed Forces Recipes would be required.

The demographic profile of the Army has changed in recent years. As of 1995, 13% of total Army personnel were female. The racial make-up included 62% white, 27% black, 5% Hispanic with the remaining 6% divided among Asian, native Indian, Alaskan Indian and other (U.S. Department of Defense, 1995). Soldiers have increased their demand for the availability of ethnic food choices in dining facilities. This is most likely a reflection of the various backgrounds of individuals entering the military and gender differences in food preferences, as well as current eating trends in the United States. The Army is therefore trying to incorporate additional ethnic-based recipes into the Armed Forces Recipe File.

In 1990, the Military Nutrition Division, USARIEM, began a collaborative effort with Pennington Biomedical Research Center (PBRC) at Louisiana State University to modify Army garrison menus. The purpose of the project was to create healthful, nutritious menu items which moderate soldiers' fat, cholesterol, and sodium intakes. New ethnic-based recipes were developed to contain decreased fat, cholesterol and sodium levels. The goals for the project were

to develop low-fat ethnic and breakfast recipes, standardize them for 100 portions and test the recipes for acceptability in an actual Army garrison.

It has been standard practice for the recipe developers at Natick Research, Development, and Engineering (RD&E) to collect food preference and acceptance ratings before new recipes are added to the Armed Forces Recipe file. Natick RD&E uses two types of test panels -

"technical" panels for quality, flavor and texture and "consumer" panels who participate in acceptance and attitude testing. Consumer panelists are asked to rate their acceptance of foods using a 9 point hedonic scale, where 9 equals "like extremely" and 1 equals "dislike extremely".

Generally a new food item must receive a mean score of at least 6.0 to be considered acceptable.

Because mean scores can be influenced by scores at either end of the spectrum, another criteria which may be used is the percentage of individuals who rate the product with a 6.0 or higher.

Jezior et al. (1990) reported that on a 9-point hedonic scale a mean of 6.0 corresponds to an acceptor size of 90% of the population. Ultimately, those products that are found acceptable under laboratory conditions are tested in actual military dining facility settings to determine whether the new food products are consumed in sufficient amounts to enter the system.

Judgments of the sensory and hedonic properties of food and food preferences are influenced by a variety of factors. Acceptability and consumption of food items depends on a complex interaction between the sensory properties of the food, the consumer expectations for it, its cognitive associations, convenience, and price (Cardello, 1993). Gender and ethnic origin also influence food preferences. In a study of food preferences in military personnel, women had higher preferences for baked potatoes, green salads and fresh fruit while men had a higher preference for grilled meat (Wyant, 1984). Researchers have documented a preference of cultures toward their own culture-specific foods (Axelson, 1986) and examined cross-cultural

flavor preferences (Meiselman & Bell, 1991). Recently a group of investigators found that just labeling a food with an ethnic title increased perceived ethnicity and acceptability of the item (Bell, 1994).

Strong relationships between food choice and attitudes have been documented, especially toward foods with a high fat content (Shepherd, 1985 & 1987). Nutritional information has been found to increase consumers' hedonic response to some products (Cheng, 1990). Solheim (1992) studied the effects of information on fat content and sensory differences on like or dislike ratings by consumers of sausage. When sensory quality was similar, false information that the fat content of the 20%-fat sausage was 12% increased the hedonic rating while correct information on fat content decreased the rating.

There are many factors that influence acceptability of a food product and the ideal situation would be to test the acceptability of a food item in the population for which it is intended, but this is not always feasible. It is possible that groups of similar age, gender and ethnic origin would evaluate food items with similar acceptability ratings. The purpose of this study was to determine if acceptability data from a young college age population was similar to acceptability scores obtained from young soldiers in an actual Army garrison setting.

METHODS

The data were collected in three phases: recipe development, acceptability testing in a young population similar to Army personnel, and acceptability testing in an Army garrison population. The first phase consisted of recipe development at PBRC by a culinary research associate and nutrient analysis using Moore's Extended Nutrient Database (MENu) (Pennington Biomedical Research Foundation, 1997).

The first phase of this project was the development of recipes, which was conducted at Pennington Biomedical Research Center (PBRC). Forty-seven new ethnic-based recipes were developed and divided into eight categories: American, breakfast, Cajun, Caribbean, Chinese, Indonesian, Italian and Mexican (Table 1). Each recipe was designated for a specific food type (bread, beef, dessert, fish, pasta, poultry, salad, starch, vegetable), as well as ethnic category. Initial testing for palatability and acceptability was conducted with a consumer panel. In addition to ethnic consideration, other criteria used for recipe development included nutritional adequacy to assure that the product was an appropriate fit for the nutrition initiatives, priced to fit within the meal cost constraints, and available for acquisition by the majority of military food service operations.

During phase two, each of the new recipes developed at PBRC were prepared as directed to yield 100 portions in the foods laboratory at Louisiana Tech University. Each of the recipes was evaluated for ease of preparation and clarity of method as outlined on the recipe cards during preparation. On 19 selected days, three new recipes per day were prepared and served in a cafeteria-like setting to individuals recruited from the campus. Subjects were able to select the food item they wished to eat from the recipes being tested for the project in a cafeteria type setting. Only foods developed for this project were included in the evaluation. For each food item selected, the subjects were asked to complete a food evaluation questionnaire.

Phase three was conducted in a military dining facility at Fort Polk, Louisiana. Over a three-week period, the new recipes were incorporated into the regular Army menu. PBRC culinary research associates were on site in the dining facility to train Army personnel in the correct techniques for preparing the new recipes. All 47 recipes were prepared and served at breakfast, lunch and dinner. Two food items from each food type which were already being

served were selected to serve as controls. For control recipes, the intent was to collect average ratings for each category so as to compare ratings for the new recipes. During the days selected for the study, evaluation questionnaires were distributed at each meal with a new menu item or a control item in the selected dining facility. These foods and their ratings are shown in Table 2. Every individual who selected a new menu item or control item was asked to complete an evaluation of the product. No information was provided regarding fat and sodium content of the recipes being evaluated.

Food Evaluation Questionnaire

The questionnaire contained closed-ended items related to demographic variables, typical use of product, and addition of condiments. In each of the three phases the recipes were evaluated with a single score for overall acceptance. The US military has a 40-year history of measuring like or dislike of food items to predict consumption. Hedonic evaluation of food started in 1950 with Peryam who developed the nine-point hedonic scale (Peryam & Pilgrim, 1957). The hedonic scale consists of nine separate phrases describing degrees of like and dislike. The scale ranges from 1 corresponding to "Dislike Extremely, to 5 "Neither Like or Dislike, and 9 "Like Extremely".

Subjects

Subjects for the first phase were faculty, staff, and students at the PBRC who volunteered to participate in routine taste tests of the recipes. They had no training in sensory analysis, but participated routinely in consumer acceptance tests of a wide range of food products. Age of the participants ranged from 18 years to about 50 years old, with 70% being younger than 29 years

of age. Approximately 50% of those testing the recipes were male, 76% were white and 12% were black.

Subjects for the second phase were a convenience sample of faculty, staff, and students at Louisiana Tech University in Ruston, LA. Individual subjects changed on a daily basis, but the composition remained fairly constant. The majority (56%) was 29 years old or less, and 44% were male. Ethnic origin as completed by the participants was 82% white and 7% black, with the remainder indicating other ethnic backgrounds.

Testing for the third phase which occurred at a Fort Polk, LA dining facility included Army personnel who regularly ate their meals in that dining facility. They were asked to participate only after they had selected a modified food item or a control item from the serving line. Therefore individuals changed from day to day, but were similar in composition to the subjects in phase two. The majority of the Army personnel (76%) were 29 years old or younger and 90% were male. Most (62%) indicated their ethnic origin as white; 18% were black; and the remaining 20% were of other ethnic origins.

Data Analysis

All analyses were performed using SAS statistical package (1995). Descriptive statistics were calculated on demographic data. Mean hedonic responses for each new recipe were analyzed separately for each of the three phases of the study by ethnic and food type. Differences in acceptability scores of new recipes between Louisiana Tech and Fort Polk were assessed using t-tests. The percentage of subjects who rated a food product on the upper end of the hedonic scale (≥ 6.0) was determined and chi square analyses were used to compare the proportion of mean ratings six and above by ethnic and food categories among the three test sites.

In order to investigate differences in food acceptability ratings with respect to test site, a logistic regression model was employed. The binary response variable consisted of the proportion of mean acceptability ratings falling into the categories of "up to six" and "six and above." The analysis was conducted on proportions because they provided more robust comparisons. Test site was used as an explanatory variable. Results were obtained by ANOVA using a general linear model procedure. In addition, contrasts were written to examine specific comparisons between test sites. Grouping the recipes into two categories, ethnic foods and food types eased interpretations of the analysis.

RESULTS

Acceptability data were compared among the test sites by overall acceptability, ethnic categories, and food type. Acceptability of recipe items was higher at the development site (PBRC) than at the validation or actual Army site (Table 3). When grouped by ethnic category, the acceptability ratings were more variable than when grouped by food type. We found ratings varied most between the development and validation sites (7.2 vs 6.6, $P < 0.05$), and least between the validation and actual Army site (6.6 vs 6.6, NS). Because ratings were similar between the validation and actual Army site, a more in-depth analysis was used to compare the two sites.

Although mean ratings varied least between the validation and actual Army setting, when t-tests were done between the two sites for 18-29 year olds, there were significant differences in ratings of breakfast foods. Four of the eight items included in the breakfast category had significantly higher ratings in the actual Army setting. There were also significant differences among dessert, salad, and starch categories. For the majority of food items, the mean scores were higher in the Army garrison setting than at Louisiana Tech (Table 3).

The percentages of acceptable (≥ 6.0) and unacceptable (< 6.0) scores for each testing site are presented in Table 4A. It is evident that the percentage of acceptable dishes was more similar between Louisiana Tech and Fort Polk than at Pennington, whose acceptability percentage was higher. Table 4B contains mean hedonic ratings for the ethnic and food type categories for 18-29 year olds at Louisiana Tech and Fort Polk. For ethnic comparisons, the only difference noted was for breakfast dishes for which the acceptability rating was higher at Louisiana Tech. A few differences were noted in food type analysis. Dessert and starch dishes were rated more acceptable at Louisiana Tech, while salad dishes were found more acceptable at Fort Polk.

Chi square analyses revealed variations in ethnic foods and food types were generally due to differences at PBRC compared with either Louisiana Tech or Fort Polk (Tables 5A and 5B). These variations were observed for all ethnic dishes except for Caribbean and Italian dishes. With respect to comparisons between Louisiana Tech and Fort Polk, significant differences were noted only in food type, specifically in pasta, poultry, salad, starches, and vegetables. Variations were noted based on the proportion of mean ratings falling into the two response acceptability categories, "up to six" and "six and above." For those contrasts that are significant, a "+" or "-" indicates the direction of the difference. For example, if the contrast of Louisiana Tech vs. Fort Polk is significant, then a "+" indicates that the proportion of ratings were significantly higher for Louisiana Tech than for Fort Polk. A "-" indicates that the proportion of ratings were significantly higher for Fort Polk than for Louisiana Tech.

DISCUSSION

The development site (PBRC) had acceptability scores differing greatly from the actual Army test site, quite possibly due to a difference in gender, age, and ethnicity of participants

evaluating the food items. The validation site (Louisiana Tech) was more closely matched to the Army site in terms of subject demographics than PBRC, thus providing scores that were similar to the Army scores.

When comparing acceptability scores of individual items between the validation and Army sites, 19 food items had scores that were different. However, only five items from those that were different at the Army site were below the 6.0 acceptability level, indicating that acceptance remained high in most items evaluated. Percentages of acceptable and unacceptable recipe items varied by site in some categories. Comparisons of food by ethnic categories revealed that the Army site was more critical of Caribbean, Mexican, and breakfast foods; comparisons of food type showed that the Army was also more critical of beef, bread, dessert, and poultry recipes.

There are several possible reasons for differences in acceptability scores of specific recipes at the validation and Army site. They could have resulted from: 1) differences in pre-preparation, holding, and serving of food; 2) types of other foods offered in combination with the test items; or 3) gender and ethnicity differences between participants at both sites. Another consideration may be food selection rates between items, given greater choice availability. Since acceptability ratings were so similar between validation and Army garrison, we anticipate that future recipe development can continue without additional testing at an actual Army garrison allowing for considerable cost savings and more timely additions to the Armed Forces Recipe File.

As a future consideration, periodic evaluations that more closely match the validation site with an Army site in demographic characteristics, such as age, gender, and ethnicity of individuals consuming the foods, would confirm the acceptability of new recipes as the

population mix of the military changes. Evaluation of newly developed recipes may also need to be conducted at alternate sites, such as Navy ships, to determine feasibility of their inclusion in the Armed Forces Recipe File for use by the other services that rely on the recipe file for meal preparation for military personnel.

REFERENCES

- Axelsson, M.L. (1986) The impact of culture on food-related behavior. *Annual Review of Nutrition*, Vol. 6, p. 345.
- Bell, R., Meiselman, H.L., Pierson, B.J. & Reeve, W.G. (1994) Effects of adding an Italian theme to a restaurant on the perceived ethnicity, acceptability, and selection of foods, *Appetite*, 22, 11-24.
- Carlson, D. E., Dugan, T.B., Buchbinder, J. C., Allegretto, J.D., & Schnakenberg, D.D. (1987) Nutritional assessment of the Ft. Riley non-commissioned officer academy dining facility. U.S. Army Research Institute of Environmental Medicine Technical Report No.T14-87.
- Cheng, H.W., Clark, A.D., & Heymann, H. (1990) Influence of selected marketing factors on consumer response to restructured beef steaks: A conjoint analysis. *Journal of Sensory Studies*, 4, 165-178.
- Cordello, A.V. (1993) New product trends: Sense or nonsense? *Cereal Foods World*, 38, 870-871.
- Jezior, B., Popper, R., Leshner, L., Greene, C. and Ince, V. (1990) Interpreting rating scale results: what does a mean mean? *Proceedings of the 32nd Annual Conference of the Military Testing Association*.
- Mielsenman, H.L. & Bell, R. (1991) Ethnic food preferences. *Proceedings of the Nutrition Society*, 50, 175.
- Peryam, D.R. and Pilgram, F. (1957) Hedonic scale method of measuring food preferences. *Food Technology*, 11(9), Supplement 9.
- Shepherd, R. & Stockley, L. (1985) Fat consumption and attitudes towards food with a high fat

- content. Human Nutrition: Applied Nutrition, 39A, 431-442.
- Shepherd, R. & Stockley, L. (1987) Nutrition knowledge, attitudes and fat consumption,. Journal of the American Dietetic Association, 87, 615-619.
- Solheim, R. (1992) Consumer liking for sausage affected by sensory quality and information on fat content. Appetite, 19, 285-292.
- Szeto, E.G., Carlson, E.E., Dugan, T.B., & Buchbinder, J.D. (1987) A comparison of nutrient intakes between a Ft. Riley contractor--operated and a Ft. Lewis military-operated garrison dining facility. U.S. Army Research Institute of Environmental Medicine Technical Report No. T2-88.
- Szeto, E.G., Dugan, T.B. & Gallo, J.A. (1989) Nutrient intakes of habitual diners in a military-operated garrison dining facility - Ft. Devens. U.S. Army Research Institute of Environmental Medicine Technical Report No. T3-89.
- U.S. Department of Defense. Military Manpower Statistics-July 31, 1995. Washington, D.C.: Washington Headquarters Services, Directorate for Information, Operations and Reports, 1995.
- Wyant, K.W., Meiselman, H.L. (1984) Sex and race differences in food preferences of military personnel. Journal of the American Dietetic Association, 84, 172-176.

Table 1. Ethnic Groupings of Developed Recipes

AMERICAN

- Apple Betty
- Garlic Cheese Potatoes
- German Potatoes
- Horseradish Potatoes
- Roasted Vegetable Salad
- Tomato Salad
- Turkey Chili
- Turkey Stroganoff
- Turnips and Greens

BREAKFAST

- Biscuits
- Breakfast Burrito
- Breakfast Potatoes
- Eggs Florentine
- Jalapeno Cheese Grits
- Oatmeal Raisin Bar
- Three Berry Muffin
- Turkey Sausage

CAJUN

- Bread Pudding
- Cajun Meatloaf
- Chicken Jambalaya
- Chicken Sauce Piquant
- Eggplant Tomato Salad
- Fish Piquant
- Red Beans with Turkey Sausage
- Summer Squash

CARIBBEAN

- Caribbean Jerk Chicken
- Caribbean Pot Roast
- Jamaican Rum Chicken
- Okra Melange

CHINESE

- Beef with Broccoli
- Chicken with Orange Glaze
- Cucumber Salad
- Oriental Chicken Salad
- Rolled Fish
- Vegetable Rice

INDONESIAN

- Fish and Mushrooms
- Thai Beef Salad

ITALIAN

- Italian Potatoes
- Pasta Primavera
- Pasta Provencal
- Pasta Putanesca

MEXICAN

- Chicken Fajitas
- Mexican Black Beans
- Mexican Cornbread
- Seven Bean Salad
- Southwestern Rice
- Vegetarian Burrito

Table 2. Acceptability ratings of control recipes (mean \pm standard deviation)

Armed Forces Recipe File Recipe Identification Number	Recipe Name	Acceptability Score (mean \pm standard deviation)
G-29	Pineapple Cake	7.5 \pm 1.8
Q-41	Peas	6.8 \pm 1.4
M-34	Macaroni Salad	6.6 \pm 1.8
L-142	Chicken	6.7 \pm 1.4
L-89	Pork Chops	7.4 \pm 1.2
L-35-2	Meatloaf with Tomatoes	7.0 \pm 1.4
E-8	Rice	6.6 \pm 1.8
Q-50	Oven Browned Potatoes	6.0 \pm 2.1
F-10	Scrambled Eggs	6.5 \pm 1.6
Q-46	Breakfast Potatoes	6.5 \pm 1.7

Table 3. Overall averages by centers for ethnic and food types

Center	Ethnic	Food Type
Pennington	7.26 ^a	7.24 ^a
Louisiana Tech	6.61 ^b	6.63 ^b
Fort Polk	6.62 ^b	6.68 ^b

^{ab} Means within columns with different superscript letters are different (P<0.05)

Table 4A. Comparison of acceptability ratings

Center	Unacceptable <6.0	Acceptable 6.0+
Pennington (PBRC)	21.3%	78.7%
Louisiana Tech	34.9%	65.1%
Fort Polk	33.2%	66.8%

Table 4B. Comparison of hedonic ratings (means \pm standard errors) for 18-29 yr olds at the test sites.

	Louisiana Tech				Fort Polk				T-test
	N	Mean	\pm	S.E.	N	Mean	\pm	S.E.	P value
<i>Ethnic</i>									
American	454	6.26	\pm	.10	162	6.14	\pm	.19	0.977
Breakfast	421	6.73	\pm	.08	164	6.18	\pm	.16	0.006
Cajun	433	7.08	\pm	.08	195	6.88	\pm	.13	0.260
Caribbean	187	7.21	\pm	.11	53	6.92	\pm	.22	0.336
Chinese	357	6.76	\pm	.10	167	6.57	\pm	.15	0.473
Indonesian	111	6.80	\pm	.13	0	--	\pm	--	--
Italian	217	7.07	\pm	.09	65	6.78	\pm	.22	0.515
Mexican	323	6.86	\pm	.10	158	6.72	\pm	.15	0.523
<i>Food Type</i>									
Bread	167	6.72	\pm	.14	95	6.33	\pm	.20	0.067
Beef	226	7.31	\pm	.09	77	7.04	\pm	.20	0.688
Dessert	115	7.28	\pm	.15	57	6.56	\pm	.27	0.025
Fish	174	6.70	\pm	.14	64	6.48	\pm	.23	0.389
Pasta	152	7.00	\pm	.11	44	7.02	\pm	.26	0.502
Poultry	558	7.19	\pm	.06	265	6.87	\pm	.11	0.087
Salad	446	6.11	\pm	.10	84	6.62	\pm	.23	0.015
Starch	524	7.05	\pm	.07	264	6.45	\pm	.13	0.002
Vegetable	132	6.02	\pm	.17	34	6.62	\pm	.33	0.082

Table 5A. Chi square analysis results for ethnic comparisons between centers.

	Chi-Square	P-value	Direction
American:			
Overall	52.37	0.0000	
Pennington vs Ft. Polk	33.41	0.0000	+
Pennington vs La. Tech	49.13	0.0000	+
La. Tech vs Ft. Polk	00.06	0.8058	
Breakfast:			
Overall	18.61	0.0001	
Pennington vs Ft. Polk	17.68	0.0000	+
Pennington vs La. Tech	12.05	0.0005	+
La. Tech vs Ft. Polk	02.20	0.1383	
Cajun:			
Overall	9.44	0.0089	
Pennington vs Ft. Polk	3.05	0.0806	
Pennington vs La. Tech	9.40	0.0022	+
La. Tech vs Ft. Polk	1.42	0.2335	
Caribbean:			
Overall	4.23	0.1206	
Pennington vs Ft. Polk	3.07	0.0799	
Pennington vs La. Tech	3.74	0.0531	
La. Tech vs Ft. Polk	0.00	0.9718	
Chinese:			
Overall	18.72	0.0001	
Pennington vs Ft. Polk	11.65	0.0006	+
Pennington vs La. Tech	18.17	0.0000	+
La. Tech vs Ft. Polk	00.23	0.6342	
Indonesian:			
Overall	20.59	0.0000	
Pennington vs Ft. Polk	14.14	0.0002	+
Pennington vs La. Tech	20.48	0.0000	+
La. Tech vs Ft. Polk	01.10	0.2951	
Italian:			
Overall	1.55	0.4614	
Pennington vs Ft. Polk	0.02	0.8917	
Pennington vs La. Tech	0.79	0.3744	
La. Tech vs Ft. Polk	1.25	0.2643	
Mexican			
Overall	10.80	0.0045	
Pennington vs Ft. Polk	08.59	0.0034	+
Pennington vs La. Tech	09.46	0.0021	+
La. Tech vs Ft. Polk	00.02	0.8957	

Table 5B. Chi square analysis results for food type comparisons between centers.

	Chi-Square	P-value	Direction
Beef:			
Overall	18.38	0.0001	
Pennington vs Ft. Polk	18.11	0.0000	+
Pennington vs La. Tech	13.05	0.0003	+
La. Tech vs Ft. Polk	01.32	0.2514	
Bread:			
Overall	1.82	0.4022	
Pennington vs Ft. Polk	1.46	0.2266	
Pennington vs La. Tech	0.14	0.7098	
La. Tech vs Ft. Polk	1.18	0.2767	
Dessert:			
Overall	8.16	0.0169	
Pennington vs Ft. Polk	8.13	0.0044	+
Pennington vs La. Tech	4.13	0.0422	+
La. Tech vs Ft. Polk	1.59	0.2072	
Fish:			
Overall	29.16	0.0000	
Pennington vs Ft. Polk	23.22	0.0000	+
Pennington vs La. Tech	28.17	0.0000	+
La. Tech vs Ft. Polk	00.10	0.7490	
Pasta:			
Overall	6.40	0.0408	
Pennington vs Ft. Polk	1.65	0.1994	
Pennington vs La. Tech	0.91	0.3392	
La. Tech vs Ft. Polk	6.39	0.0115	-
Poultry:			
Overall	23.32	0.0000	
Pennington vs Ft. Polk	21.83	0.0000	+
Pennington vs La. Tech	05.17	0.0230	+
La. Tech vs Ft. Polk	10.27	0.0013	+
Salad:			
Overall	68.15	0.0000	
Pennington vs Ft. Polk	06.42	0.0113	+
Pennington vs La. Tech	61.41	0.0000	+
La. Tech vs Ft. Polk	20.70	0.0000	-
Starches:			
Overall	8.36	0.0153	
Pennington vs Ft. Polk	7.90	0.0049	+
Pennington vs La. Tech	1.52	0.2174	
La. Tech vs Ft. Polk	4.04	0.0445	+
Vegetables:			
Overall	29.77	0.0000	
Pennington vs Ft. Polk	01.33	0.2495	
Pennington vs La. Tech	25.57	0.0000	+
La. Tech vs Ft. Polk	10.41	0.0013	-

APPENDIX VII
ENHANCING MILITARY DIETS

Food Preference Questionnaire Final Values

2569 total records were collected from nine military bases.

Records collected by Military Branch			
Branch	Male	Female	Total
Army	693	270	969
Air Force	327	103	438
Navy	604	88	698
Marines	450	14	464

Records collected by Military Base			
Base	Male	Female	Total
USS Artic	111	31	142
Sam Houston	298	221	519
Ft. Lewis	115	0	115
USS Montpelier	25	0	25
Keesler AFB	237	91	334
Ft. Bragg	280	49	335
Barksdale AFB	90	12	104
Camp Lejune	450	14	464
Pennsacola	468	57	531

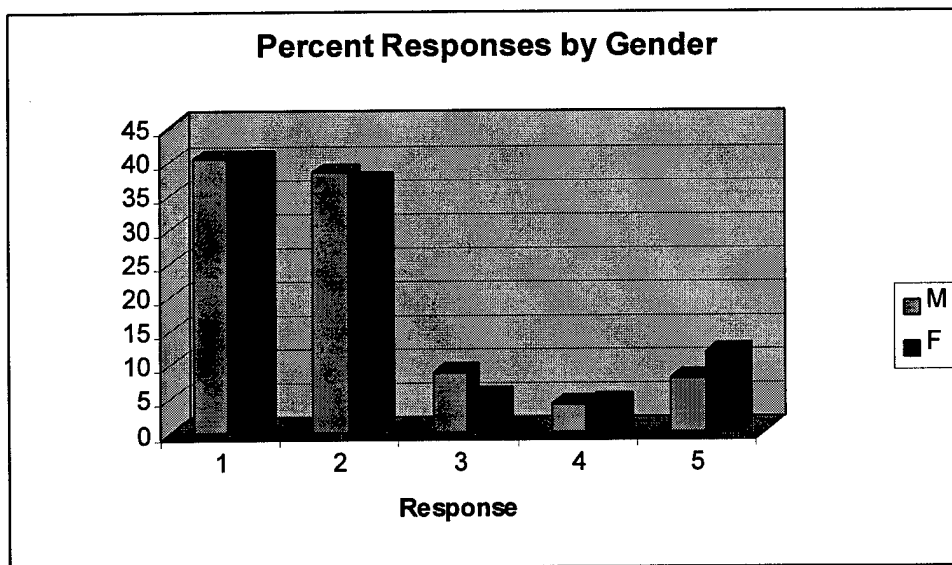
Ethnic Origin by Gender			
Ethnicity	Male	Female	Total
Caucasian	1281	257	1538
African American	314	127	441
Asian	73	12	85
Hispanic/Mexican American	228	47	275
American Indian	34	3	37
Other	121	26	147
Grand Total	2051	472	2523

Fast Food Consumption

Total Respondents by Branch and Gender			
Branch Name	Male	Female	Total
Air Force	315	101	416
Army	680	265	945
Marines	435	13	448
Navy	575	87	662
Grand Total	2005	466	2471

Percent Responding per Each Choice by Branch and Gender						
Branch	Gender	1	2	3	4	5
Air Force	M	41.90	34.92	8.25	5.40	9.52
	F	41.58	34.65	3.96	4.95	14.85
Army	M	40.00	40.00	9.12	3.97	6.18
	F	35.09	40.75	6.04	5.28	11.70
Marines	M	30.34	43.68	12.18	6.67	7.13
	F	46.15	23.08	7.69	15.38	7.69
Navy	M	47.30	34.09	6.61	2.09	9.91
	F	54.02	31.03	5.75	0	9.20

Percent Responding per Each Choice by Gender					
Gender	1	2	3	4	5
M	40.3	38.3	8.93	4.24	7.98
F	40.34	37.12	5.58	4.51	11.8

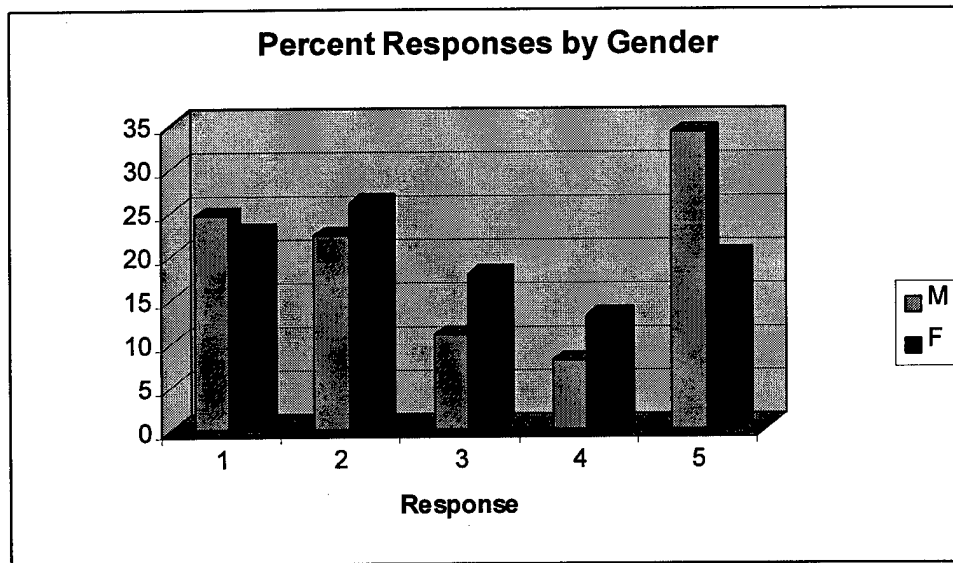


Diet Food Consumption

Total Respondents by Branch and Gender			
Branch Name	Male	Female	Total
Air Force	314	100	414
Army	667	262	929
Marines	423	13	436
Navy	563	86	649
Grand Total	1967	461	2428

Percent Responding per Each Choice by Branch and Gender						
Branch	Gender	1	2	3	4	5
Air Force	M	25.16	21.34	10.19	9.87	33.44
	F	30.00	30.00	15.00	10.00	15.00
Army	M	26.69	23.09	10.04	8.10	31.78
	F	20.61	23.28	18.32	14.89	22.14
Marines	M	20.57	25.53	13.71	5.91	34.28
	F	15.38	23.08	46.15	0	15.38
Navy	M	24.87	19.18	10.30	8.17	37.30
	F	22.09	30.23	15.12	12.79	19.77

Percent Responding per Each Choice by Gender					
Gender	1	2	3	4	5
M	24.61	22.22	10.93	7.93	34.16
F	22.78	26.03	17.79	13.02	19.96

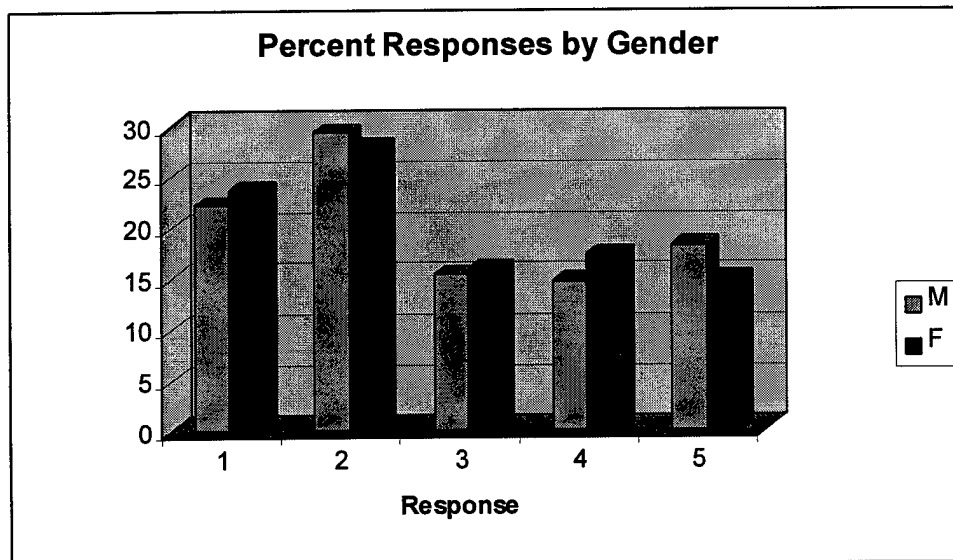


Heart Healthy Food Consumption

Total Respondents by Branch and Gender			
Branch Name	Male	Female	Total
Air Force	314	100	414
Army	673	264	937
Marines	430	13	443
Navy	567	83	650
Grand Total	1984	460	2444

Percent Responding per Each Choice by Branch and Gender						
Branch	Gender	1	2	3	4	5
Air Force	M	25.16	23.89	13.69	16.88	20.38
	F	27.00	34.00	13.00	14.00	12.00
Army	M	22.44	29.72	18.42	15.45	13.82
	F	23.11	25.76	16.67	19.32	15.15
Marines	M	18.84	33.72	14.88	13.95	18.60
	F	23.08	38.46	23.08	0	15.38
Navy	M	22.75	28.75	13.23	13.23	22.05
	F	21.69	26.51	15.66	18.07	18.07

Percent Responding per Each Choice by Gender					
Gender	1	2	3	4	5
M	22.18	29.39	15.42	14.72	18.25
F	23.70	28.04	15.87	17.39	15.00

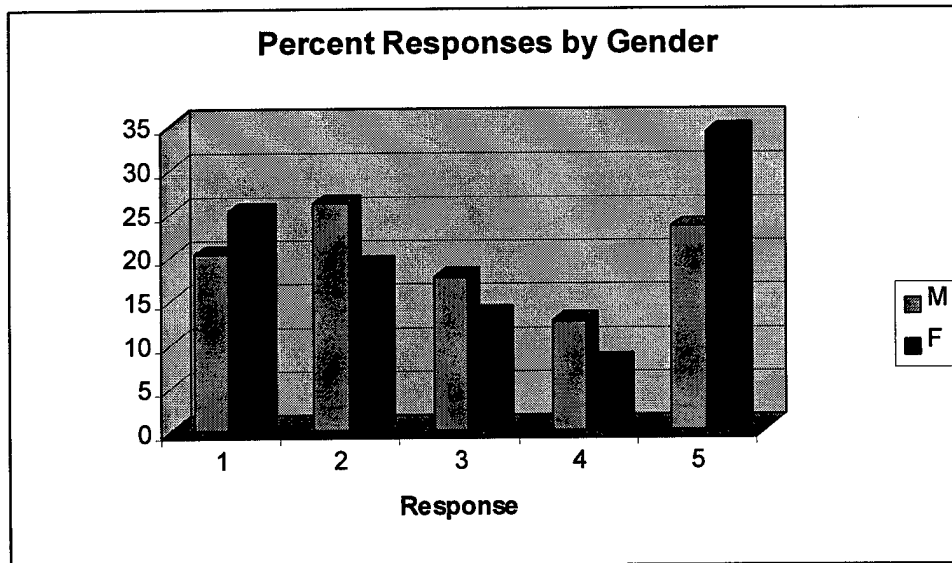


Energy Food Consumption

Total Respondents by Branch and Gender			
Branch Name	Male	Female	Total
Air Force	313	99	412
Army	674	263	937
Marines	424	13	437
Navy	562	84	646
Grand Total	1973	459	2432

Percent Responding per Each Choice by Branch and Gender						
Branch	Gender	1	2	3	4	5
Air Force	M	22.36	23.32	14.06	11.18	29.07
	F	28.28	20.2	15.15	4.04	32.32
Army	M	20.33	30.56	20.18	12.17	16.47
	F	21.67	20.15	14.83	8.37	34.22
Marines	M	17.92	23.82	21.23	14.62	22.41
	F	30.77	15.38	7.69	7.69	38.46
Navy	M	20.46	24.2	13.88	12.28	29.18
	F	32.14	14.29	7.14	10.71	35.71

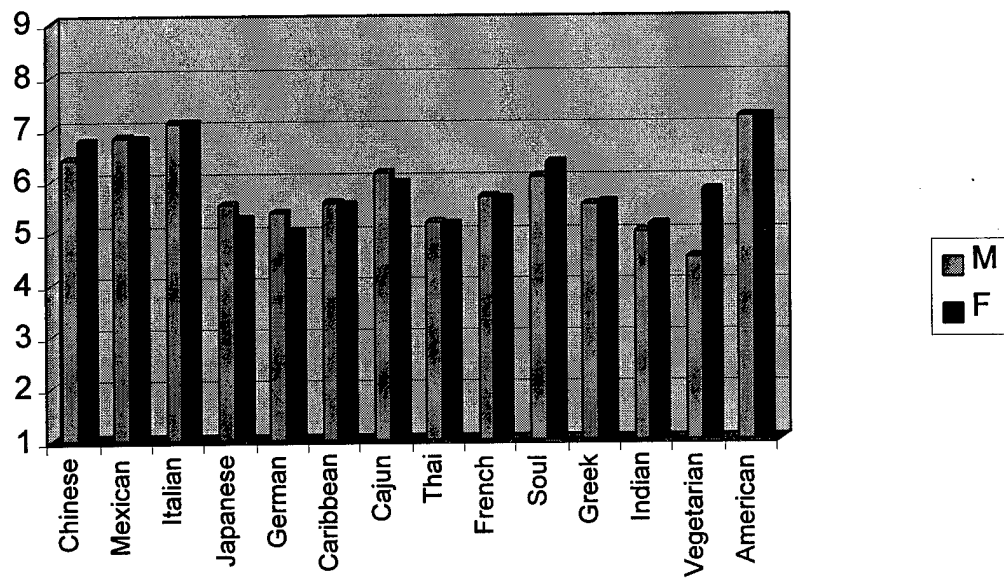
Percent Responding per Each Choice by Gender					
Gender	1	2	3	4	5
M	20.17	26.15	17.64	12.57	23.37
F	25.27	18.95	13.29	7.84	34.2



Hedonic Ratings of Specialty Foods

Average Ratings		
Food Type	M	F
Chinese	6.4	6.8
Mexican	6.8	6.8
Italian	7.1	7.1
Japanese	5.5	5.3
German	5.4	5.0
Caribbean	5.6	5.5
Cajun	6.1	6.0
Thai	5.2	5.1
French	5.7	5.6
Soul	6.1	6.3
Greek	5.5	5.6
Indian	5.0	5.2
Vegetarian	4.5	5.8
American	7.2	7.2

Hedonic Rating of Speciality Foods



Average Hedonic Rating of Various Foods

Food	Male	Female
Apple Pie	7.2	7.1
Bagels with Cream Cheese	6.7	7.5
Baked Potato	7.0	7.5
Baked Stuffed Flounder	6.0	5.7
Barbecued Ribs	7.0	6.5
Beef and Potato Stew	6.7	6.1
Breakfast Muffins	6.7	7.3
Broccoli and Cheese Stuffed Baked Potato	6.5	7.4
Broiled Lemon Fish	6.0	6.0
Cake with Frosting	6.6	6.8
Chicken Fried Steak with Gravy	6.9	6.2
Chicken Gumbo	6.5	6.2
Chicken Noodle Soup	6.9	7.0
Chicken Pasta Salad	6.6	6.7
Chicken Quesadillas	7.1	7.3
Chocolate Chip Cookies	7.3	7.7
Clam Chowder	6.1	5.8
Creamed Beef on Toast	5.5	4.5
Creole Snapper in Spicy Tomato Sauce	5.7	5.1
Fettuccini Alfredo	7.2	7.4
French Fries	7.3	7.4
Fried Fish Fillets	6.0	5.4
Fried Okra	5.4	5.3
Fried Pork Chops	6.4	5.9
Fruit Salad	7.1	7.6
Garden Vegetable Lasagna	6.2	7.0
Granola	6.6	6.8
Greek Vegetarian Pasta	5.8	6.5
Green Bean Casserole	5.4	5.6
Green Salad	7.0	7.4
Hamburger	7.2	6.8
Hash Browned Potatoes	7.1	7.0
Herb Roasted Chicken	7.0	7.0
Honey Baked Ham	6.8	6.3
Lasagna	7.8	7.8
Lemon Meringue Pie	6.2	5.9
Meatless Beans over Rice	5.8	6.0
Minestrone	5.8	5.9
Oatmeal	6.0	6.3
Oriental Beef Stir Fry	6.7	6.6
Pancakes	7.0	7.3
Pasta with Tomato Sauce	7.1	7.4
Peach Cobbler	6.7	6.8
Potato Salad	6.2	6.6

Food (cont.)	Male	Female
Pudding	6.8	7.0
Roast Beef	7.1	6.4
Scalloped Potatoes	6.5	6.6
Scrambled Eggs	6.8	5.9
Smothered Chicken and Rice	6.9	6.8
Southern Fried Chicken	7.2	7.0
Southern Style Greens	6.1	6.4
Spaghetti with Meat Sauce	7.6	7.3
Spicy Buffalo Wings	7.3	6.7
Spicy Mexican Chili	6.8	5.9
Spicy Spanish Rice	6.5	6.2
Southwestern Taco Salad	7.0	7.0
Spinach and Artichoke Dip	5.0	5.2
Steamed Vegetables	6.3	7.1
Stir Fried Rice	6.8	7.4
Stuffed Pork Roast	6.3	5.2
Tuna Casserole	5.6	5.7
Veggie Burger	4.4	5.5

APPENDIX VIII

STRESS, NUTRITION AND IMMUNE FUNCTION LABORATORY

Fig 1. Effect of Dietary Fat on Proliferation

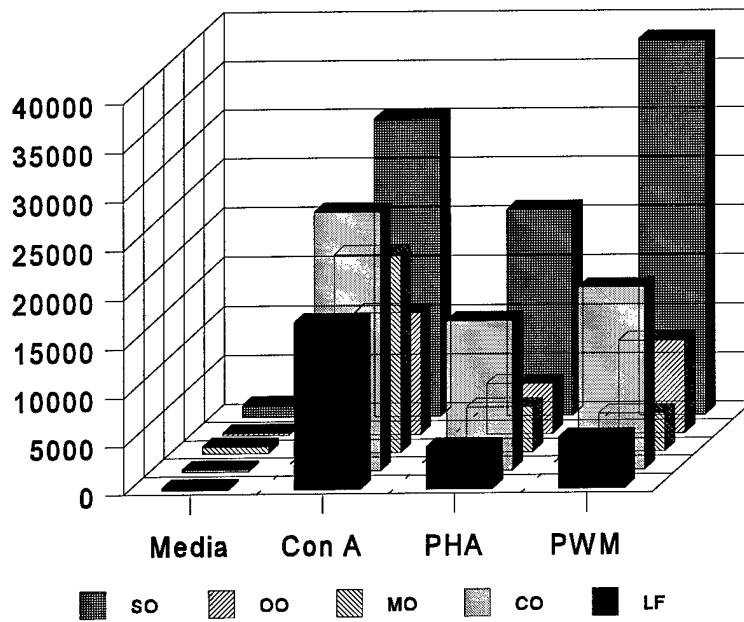


Fig 2. Effect of Dietary Fat and FCS on Lymphoproliferation

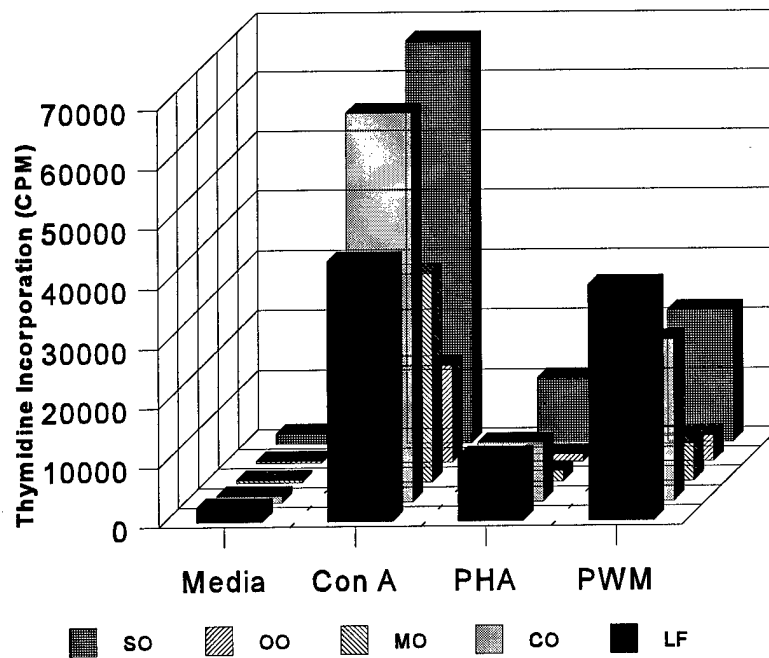
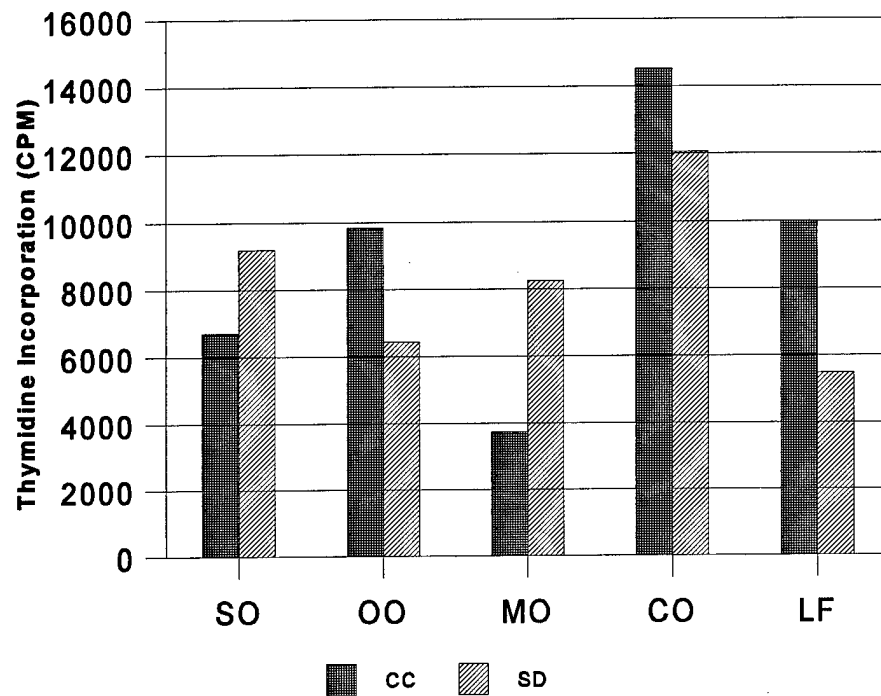


Fig 3. Effect of Dietary Fat on SD-induced Immunomodulation



APPENDIX IX
METABOLIC UNIT PROJECT

NONE